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Effect Of Artesunate On The Nissl Bodies Of The Cerebellum Of Wistar Rats

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ABSTRACT

The study was to assess the effect of the administration of different doses of artesunate on the Nissl bodies of the cerebellum of Wistar rats. Twenty adult albino Wistar rats weighing between 160-180g were equally assigned into four groups (A, B, C and D). Group A served as the control that received distilled water, while groups B, C and D were the experimental groups. Groups B and C received 2.86mg/kg and 5.71mg/kg of artesunate respectively for three days, while group D received 2.86mg/kg of artesunate for six days. Twelve hours after the last administration the animals were sacrificed. There was reduced staining intensity of Nissl bodies in groups B, C and D compared to the control. The reduction in staining was more in groups C and D especially in the granular and Purkinje cortical layers. These results revealed that artesunate cause reduced Nissl bodies of the cerebellum of Wistar rats, and these reductions were dose and time dependent.

Key Words: Artesunate**,** cerebellum, Nissl bodies, Wistar rats

Artemisinin is a sesquiterpene lactone isolated from *Artemisia annua*, a herb that has traditionally been used in China for the treatment of malaria. Artemisinins and its derivatives are the newly introduced drugs in this part of the world. They act rapidly on parasites and do not remain in the blood stream for long. Therefore most parasites are destroyed before the drug concentrations drop to the sub-therapeutic levels, reducing the chances that parasites will form resistance (WHO 2001).

Artesunate (AS) is a water-soluble hemisuccinate derivative of artemisinin. AS and its active metabolite, dihydroartemisinin are potent blood schizonticides, active against the ring stage of the parasite. It is ideal for the treatment of severe malaria, including cerebral malaria. It is also active against chloroquine and mefloquine resistant strains of *P. falciparum* (Ipca 2004). It is made up of endoperoxide bonds that produce carbon-centered free radicals. Release of these active oxygen species from this bond kills the parasite if accumulated in the erythrocytes (Meshnick 2002). It also suppresses the production or activity of antioxidant enzymes in the erythrocytes, causing lysis of the parasitic cell due to the highly reactive free oxygen radicals (Ipca 2004). AS has been shown to cause significant toxic effects; Neurotoxicity has been observed in animal studies but not in humans (Gordi and Lepist 2004). These include; neurologic defects such as gait disturbances, loss of spinal and pain reflexes (Dayan 1998), prominent loss of brain stem and eye reflexes and prominent neuropathic lesions sharply limited to the brain stem (Genovese *et al*, 2000 and Nontprasert *et al*, 2002). Ataxia and slurred speech (Miller and Panosian 1997) have been reported with artesunate. The above neuronal dysfunction may have been due to the effect of the drug on the cerebellum, since the cerebellum control fine movement and balance. Therefore this study was carried out to check the effect of the drug on the cerebellum of Wistar rats.

MATERIALS AND METHODS

Twenty adult Wistar rats weighing between 150-180g were procured from the Department of Anatomy Animal House, University of Calabar, Calabar. Random sampling was used to divide the rats into four groups of five animals each. Two packets of artesunate (AS) were procured from a reputable pharmacy in Calabar. Each packet of the drug contained twelve blistered tablets of 50mg of AS per tablet. Two tablets of AS containing 50mg each is recommended per day. Thus, 100mg of AS is taken by a physiologic man (70kg) per day. Converting it to the dosage of rats (200g), 2.86mg/kg is its equivalent. The animals in group A

(the control) were administered with distilled water. Groups B and D animals were treated with 2.86mg/kg of AS each for three and six days respectively, while group C animals were treated with a double dose (5.71mg/kg of AS) for three days. The treatments was twelve hourly. Table 1 shows the treatments.

Twelve hours after the last administrations the animals were sacrificed. The brains were extracted by opening of the skull to have assess. The whole brains were preserved using formal saline. When properly fixed, the cerebellums were excised. Routine process using cresyl fast violet for the Nissl bodies was carried out (Lowe and Cox 1992).

Table 1: The administration of the drugs.

Group (n=5)	Dosage per day	Duration (day)
A	Control	3
B	2.86 mg/kg	3
C	5.71 mg/kg	3
D	2.86 mg/kg	6

RESULTS

The photomicrograph of the control group (group A) showed the three cortical layers and the medullary ray deep to them. The Nissl bodies are well stained in the three cortical layers making the neurons easily distinguishable. Group B treated with 2.86 mg/kg of artesunate for three days showed the staining intensity of the Nissl substances which appeared as to the control. Group C treated with 5.71 mg/kg of artesunate for three days showed the staining intensity of the Nissl bodies being reduced in the granular and Purkinje cortical layers, group D treated with 2.86 mg/kg of artesunate for six days showed reduced staining of the Nissl substances in the granular and Purkinje cortical layers. The colour intensity profile is represented in Table 2.

Table 2: Nissl substances colour intensity profile

Group	Nissl Colour Intensity	
A	+++	
B	+++	
C	++	
D	-	Less present

+++ - Highly present
++ - Present
+ - Less present

DISCUSSION

Nissl substances are composed of rough endoplasmic reticulum in the neurons, and are responsible for synthesizing proteins. They are prominent in neurons where they are mostly located in the soma (cell body) and extend along the dendrites but do not penetrate axons (Snell 2001). This makes them an important index in tracing neuronal population (Singh 2002). Nissl substances appear like fine dust particles when stained for light microscopic study. They contain particle of ribonucleic acid (RNA) arranged in a manner similar to that in secretory cells and is concerned with protein synthesis. Neurons require large amount of proteins to maintain functional integrity, the protein are utilized or incorporated in the synthesis of trophic substances, enzymes and neurotransmitters (Lowe and Cox 1992).

Certain stains are applied for the demonstration of Nissl substances. These stains are used routinely in neurohistology, not so much to demonstrate Nissl substances, but as a stain to show cellular pattern (Lowe and Cox 1992). In this

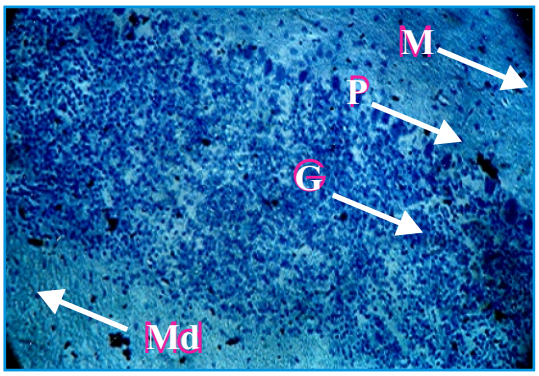

Fig 1:The control group shows the three cortical layers and the medullary ray deep to them. (M= Molecular layer, P= Purkinje layer, G= Granular layer, Md= Medulla). Mag x400

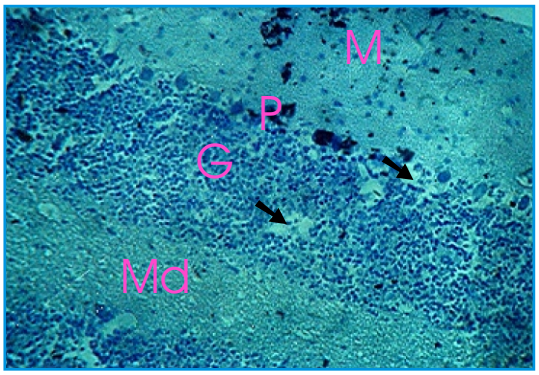

Fig 2:Group B shows the staining intensity of the Nissl bodies which appears normal compared to the control. Mag x400

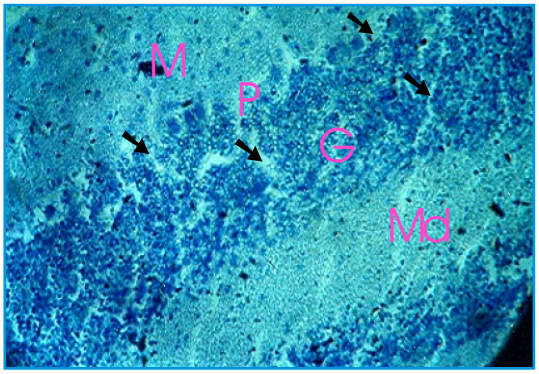

Fig 3:Group C shows the staining intensity of the Nissl substances being reduced in the granular and Purkinje cortical layers. Mag x400

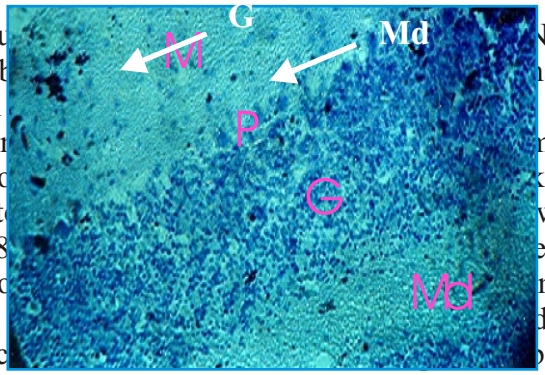

Fig 4: Group D shows reduced staining intensity of the Nissl substances in the granular and Purkinje cortical layers. Mag x400

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fluid, due to increased dose in dosage and time.

AS releases alkylating free radicals from its endoperoxide bond (Robert *et al* 2001, Meshnick 2002), which may have caused the reduction in Nissl substance staining seen in this study.

The Nissl substances fragment or disintegrate in degenerating cells following injury and tend to disappear in severe injury or from cells which have been kept in a state of activity for a prolonged period (Lowe and Cox 1992). The disappearance of the Nissl substance may be due to chromatolysis. Chromatolysis is the migration of the Nissl substances toward the periphery of the soma due to either trauma or due to other exogenous agents (Lowe and Cox 1992, Snell, 2001). This usually results in loss of functions of the Nissl substances, as well as, loss of staining. Thus, the loss of function of the Nissl substances may result in the loss of the protein synthesizing ability of the neurons, and since protein is the working molecules of the cells, this may ultimately result in death of the cells.

This result shows that artesunate caused Nissl substances reduction especially in the groups treated with excess of the drug. In conclusion, artesunate may cause reduction in Nissl substances distribution if proper adherence to its prescribed dosage is not followed.

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