Chemistry Research Journal, 2018, 3(4):98-104

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Effects of Nifedipine on the Histology of the Cerebella Cortex of Adult Male Wistar Rats

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Abstract Hypertension is a major public health problem due to its high prevalence, and is a major risk factor for cardiovascular diseases and other complications as well. Nifedipine, a calcium channel blockers is used to manage this condition. The study is to investigate the effects of oral administration of nifedipine on the histology of the cerebella cortex of adult male Wistar rats. Twenty adult male Wistar rats, weighing 160g - 200g were divided into four groups (n = 5). Group 1 served as the control, while groups 2 - 4 were the test groups, and administered 1.0 mg/kg, 1.8 mg/kg and 3.6 mg/kg body weight of nifedipine, respectively daily for 30 days. At the end of the experiment, the rats were sacrificed and the cerebella were excised and routinely processed for histology, using haematoxylin and eosin, as well as Cresyl violet stains. Results of the Cerebella cortical histology showed mild histopathology in the 1.0 mg/kg nifedipine group, while there were hypertrophied cells in the 1.8 mg/kg and 3.6 mg/kg nifedipine groups with significantly ($p \leq 0.05$) less cell population in these test groups. Nissl substance was less stained in the 1.0 mg/kg, 1.8 mg/kg and 3.6 mg/kg nifedipine groups, with significantly ($p \leq 0.05$) less distribution in these test groups, all compared with control group. In conclusion, the results of this study showed that nifedipine administration may result in adverse cellular adaptations and loss of neurons especially at higher dosages. This may result in the functional impairment of the cerebellum.

Keywords Nifedipine, histology, cerebella cortex, Nissl substance, Wistar rat

Introduction

Hypertension is a major public health problem due to its high prevalence around the globe [1-3]. Around 7.5 million deaths or 12.8% of the total of all annual deaths worldwide occur due to high blood pressure [4], and is predicted to increase up to 1.56 billion adults with hypertension by 2025 [5]. Hypertension is a major modifiable risk factor, which significantly and independently increases the risk of developing major cardiovascular, cerebrovascular and renal complications [6]. On the other hand, an effective treatment of hypertension substantially reduces the risk of developing such complications and improves cardiovascular prognosis [7].

One such treatment drugs, belonging to the class of calcium channel blockers is nifedipine. It is indicated in the treatment of hypertension, chronic coronary ischemia, and/or supraventricular arrhythmias, and is safe and effective in reducing hard cardiovascular end points. The most common side effects associated with nifedipine like other calcium channel blockers are vasodilation and non-volume-dependent form of peripheral edema, flushing, and headache. Despite the sometimes discomforting side effects seen with calcium channel blocker therapy, their robust

blood pressure-lowering effect makes them an important component of most multidrug regimens used for blood pressure control [8].

Nifedipine acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, nifedipine prevents calcium-dependent myocyte contraction and vasoconstriction. It is reported that nifedipine treatment reduces ischemic lesion volume after cerebral ischemia possibly because of decrease in oxidative stress with an increase in antioxidant activities within the ischemic area [8]. Furthermore, nifedipine has been reported to be safe and more effective in controlling blood pressure in severe pre-eclampsia [9].

Different researches have shown diverse effects of nifedipine: it induces histological changes in the parotid gland of hypertensive rat [10]; it enhances the survival of axotomized substantia nigra neurons [11]; and affects sperm count and motility, but not the testis [12]. However, there are no reports on the cerebella effect of this drug, which warranted this study.

The cerebellum is an important brain area vital in skilled motor control and balance, and is made up of three distinct layers; molecular, Purkinje cell and granular layers, having five major neuronal types [13]. In view of the reported adverse effects of nifedipine on other sensitive body tissues, this study investigated the histological effects of the cerebella cortex in male adult Wistar rats.

Materials and Methods

Twenty adult male Wistar rats weighing between 160 - 200 g were obtained and were allowed to acclimatize for one week in the Experimental House of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus. They were fed daily with growers mesh (Sander Feeds) and water *ad libitum*.

Experimental Design

The animals were randomly divided into four groups: Group 1 was the control, and were administered 5.0 ml/kg of distilled water; Groups 2 - 4 were the test groups and were administered 1.0 mg/kg, 1.8 mg/kg, and 3.6 mg/kg body weight of nifedipine, respectively. The drug administration was orally and thrice daily for 30 days.

Animal Sacrifice

The rats were sacrificed twenty four hours after the administrations using cervical dislocation. The cerebellum were excised and processed routinely for histological studies, using haematoxylin and eosin (H & E) and Cresyl fast violet stains. Cell counts of the sections were carried out with the aid of ImageJ® software. Statistical analysis using GraphPad Prism was done for one-way analysis of variance and post hoc Tukey's Multiple Comparison test. Data were regarded as significant at $p \leq 0.05$.

Results and Discussion

This study investigated the effects of nifedipine on the cerebella cortical histology of adult male Wistar rats. The cerebella cortex which is the external layer of the cerebellum maintains equilibrium, coordinates muscles' tone, and is involved in learned memory [14]. These functions are germane in the normal functioning of animal forms, and any alteration may be detrimental. The Purkinje and granule cells of the cerebellum have been identified as the most important targets of toxic substances [15], which warranted this study.

In the present study, the section of the cerebella cortex of the control group showed three cortical layers, namely; the molecular, Purkinje and granular. There was no apparent histopathology of the sections, with normal sparse cellular distribution in the molecular layer, while the Purkinje and granular layers contained Purkinje and granular-shaped cells respectively. Within the granular layer were glomeruli interspaced between the granular and Golgi cells (Figure 1 and 2). The section of the cerebella cortex of the group that received 1.0 mg/kg body weight of nifedipine showed prominent Purkinje neurons compared with the control group. The section of the cerebella cortex of the group that received 1.8 mg/kg body weight of nifedipine showed hypertrophied cells compared to the control group. The

section of the cerebella cortex of the group that received 3.6 mg/kg body weight of nifedipine showed cellular hypertrophy including the Purkinje cells compared to the control group (Figure 2).

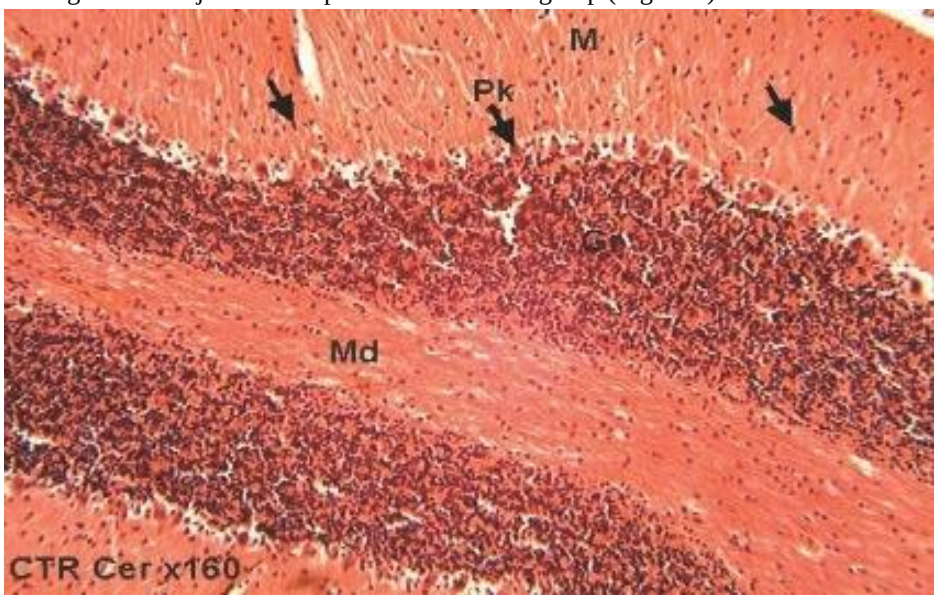

Figure 1: The section of the cerebella cortex of the control group showing the molecular (M), Purkinje (Pk), and granular layers (Gr). The molecular layer is the outermost layer which contains few nerve cells. The Purkinje cell layer is the middle layer that contains a monolayer of Purkinje cells sandwiched between the molecular and granular layers. The granular layer is the inner layer that consists of densely packed granule cells. Deep to the granular layer is the medulla (Md) consisting of mostly axons and few neuronal bodies, but numerous glia. Arrows indicate neuronal cell bodies. ×160, H&E

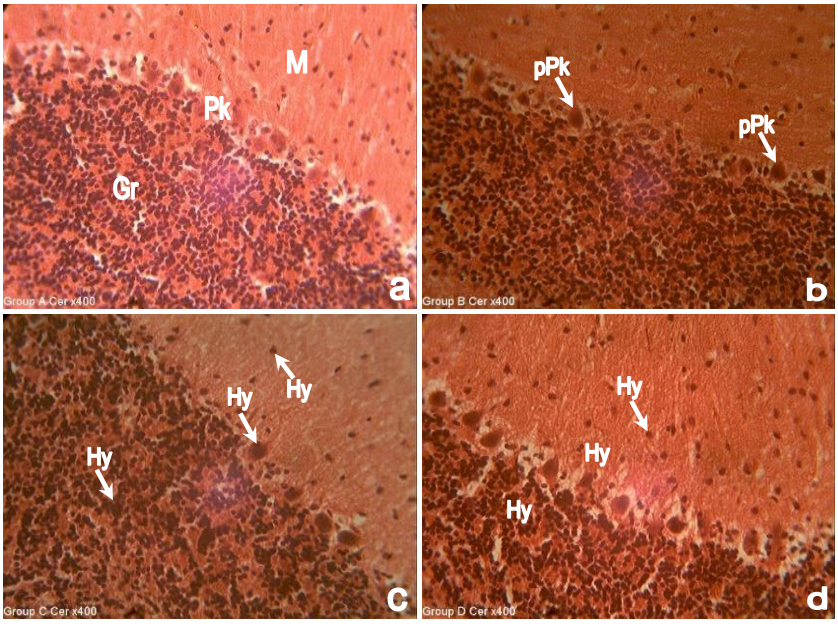

Figure 2: Photomicrographs of the section of the cerebella cortex of the control and test groups showing the molecular (M), Purkinje (Pk), and granular layers (Gr). ×400, H&E

i. The control shows no apparent histopathology.
ii. The 1.0 mg/kg nifedipine group shows prominent Purkinje cells (pPk)
iii. The 1.8 mg/kg nifedipine group shows hypertrophied cells (Hy).

iv. The 3.6 mg/kg nifedipine group shows hypertrophied cells (Hy).

Cellular hypertrophies are changes that arise due to trauma to the cells [16]. These morphological alterations to the cellular integrity may either be physiology or pathological [17]. In physiological conditions, the cellular changes may be reversed when the causative agent is withdrawn [18]. However, in pathological conditions the changes may be irreversible leading to neuronal degeneration and resulting in extensive neuronal death in the central nervous system [18,19]. Nifedipine has been reported to cause biochemical or morphological changes [10,20], and this may have played out in the present study.

In the present study, there were significantly ($p \leq 0.05$) less cell population in these test groups compared with the control group (Figure 3). These less cell population may be due to cell degeneration as previously suggested. Cell degeneration may occur either by necrosis or apoptosis. Necrosis affects extensive cell population which involves cytoplasmic swelling, while apoptosis is an organized form of self-destruction that is characterized by cell shrinkage [21]. Both cell death processes could be induced by cytotoxic drugs or physical stimulation [22]. This report may have been a case in the present study.

One conspicuous feature in the perikaryon of neurons is the macromolecular structure known as Nissl substance. It is rich in DNA and composed of stacks of rough endoplasmic reticulum and intervening groups of free ribosomes [23]. The neuron is one of the most complex cell in the body and since it is incapable of dividing after the first few days of life, loss of neurons is irreversible. Nissl substance can be identified using special stain such as the Cresyl fast violet and because Nissl substance, located in the neuronal perikaryon may serve as a marker of neurons [24]. Disappearance or less staining of Nissl substance have been observed in injured neurons, a process known as chromatolysis [24,25].

In the present study, the section of the cerebella cortex of the control group showed numerous well stained neurons with Nissl substance. The section of the cerebella cortex of the group that received 1.0 mg/kg body weight of nifedipine showed slightly less stained Nissl substance compared with the control. The section of the cerebella cortex of the group that received 1.8 mg/kg body weight of nifedipine showed less stained Nissl substance. The section of the cerebella cortex of the group that received 3.6 mg/kg body weight of nifedipine showed less stained Nissl substance compared with the control (Figure 4). There were significantly ($p \leq 0.05$) less cerebella cortical Nissl substance distribution of the test and compared with control group. Also there was significantly ($p \leq 0.05$) less cerebella cortical Nissl substance distribution in the 1.8 mg/kg and 3.6 mg/kg body weight nifedipine groups compared with the 1.0 mg/kg body weight nifedipine group, while there was no difference ($p > 0.05$) in the 1.8 mg/kg and 3.6 mg/kg body weight nifedipine groups (Figure 5).

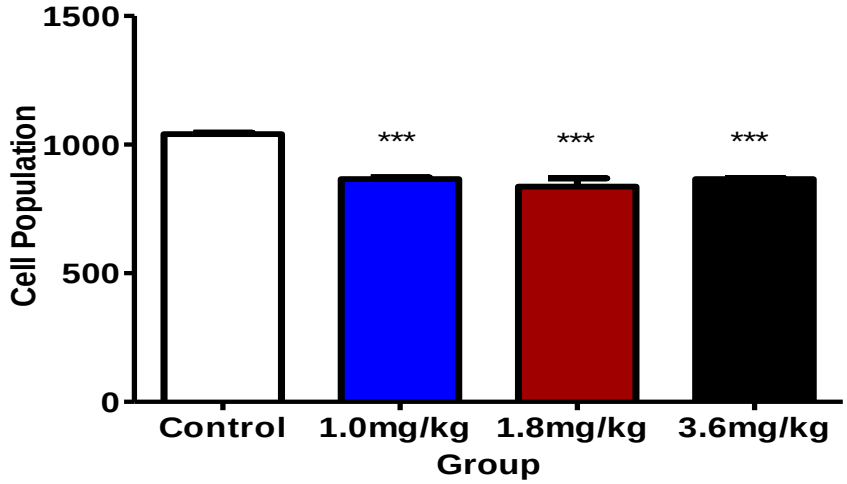

Figure 3: Cerebella cortical cell population of the test and control groups
*** *Significantly at $p \leq 0.05$ less compared with control group*

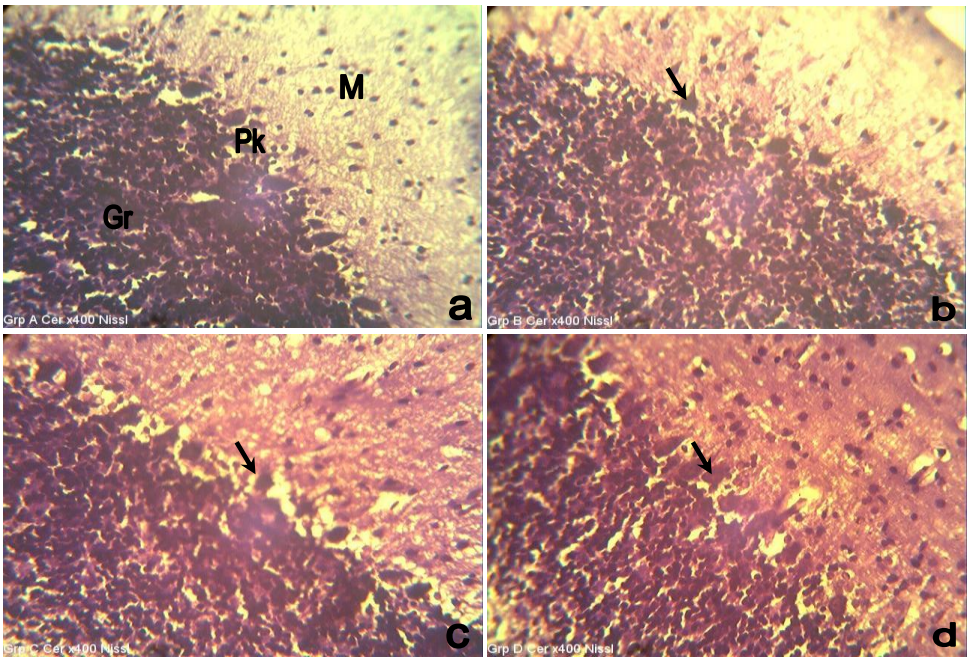

Figure 4: Photomicrographs of sections of the cerebella cortex of control and test groups showing Nissl distribution. Cresyl fast violet, ×400

i. The control group show well stained Nissl susbstance
ii. The 1.0 mg/kg nifedipine group shows slightly less stained Nissl substance (arrow).
iii. The 1.8 mg/kg nifedipine group shows less stained Nissl substance (arrow).
iv. The 3.6 mg/kg nifedipine group shows less stained Nissl substances (arrow).

M= Molecular layer, Pk=Purkinje layer, Gr=Granular layer.

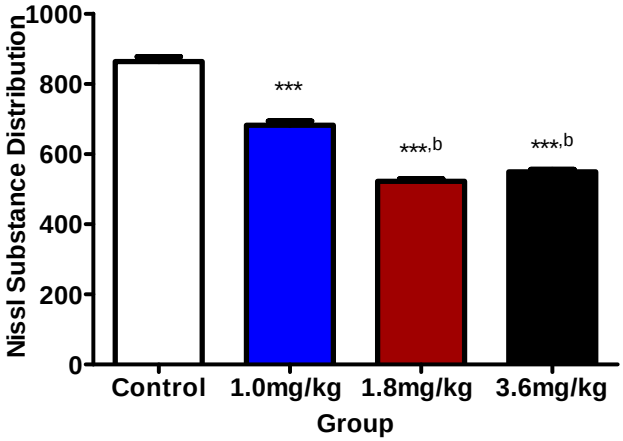

Figure 5: Cerebella cortical Nissl distribution in the test and control groups

**** Significant less at $p \leq 0.05$ compared with control group*

****,b Significant less at $p \leq 0.05$ compared with the 1.0 mg/kg body weight nifedipine group*

The reduced staining and population of neurons with Nissl substance may be due to chromatolysis which usually results in migration of the Nissl towards the periphery of the soma following neuronal injury [24,25]. Ajibade et al. [26] in a study on microstructural observation of Nissl substance in cerebella cortex of adult Wistar rats following quinine administration observed less staining intensity of Nissl substance. Muonagolu and Ekong [27] also reported loss of Nissl substance in the prefrontal cortex of Wistar rats exposed to *Allium sativum.* The reduction in number of

neurons with Nissl substance may affect the synthesis of protein with correlation with functions. Chemical and toxic substances affect Nissl substances and influence their metabolic activity [28].

It is reported that chemically induced changes is usually characterized by different patterns of neuronal cell injury and even death [29]. The Purkinje cell, which is the only efferent of the cerebella cortex, is more vulnerable to injury and death when there is mechanical lesion of the cerebellum. This cell will eventually disrupt the activities of the cerebellum, viz a viz the brain and the body at large.

Conclusion

The results of this study showed that nifedipine administration may result in adverse cellular adaptations and loss of neurons especially at higher dosages. This may result in the functional impairment of the cerebella cortex.

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