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Exogenous melatonin restored the cyto-architectural integrity and biochemical activities of the cerebrum in sodium fluoride induced toxicity

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SUMMARY

The cerebrum is responsible for motor, sensory and autonomic activities of the human body, and it is believed that fluoride exposure to the biological system can impede these functions. Therefore, it is imperative to introduce melatonin to limit the extent of fluoride toxicity on the cerebrum and understand the mechanism involved in the aforementioned process. Thirty-two rats were randomly selected into 4 groups (n=8, per group). Groups I-IV received oral administration of 0.2ml of normal saline (NS), 500ppm of sodium fluoride (NaF), concurrent administration of sodium fluoride and melatonin (NaF+MLT), and sodium fluoride before melatonin (NaF-MLT) for fourteen days respectively. At the end of these treatments, the rats were euthanized and cerebral tissues were excised for histological, histochemical and biochemical analyses. Sodium fluoride distorted the shapes and size of the cells and caused constriction of the blood vessels, as well as presence of vacuolations in the cells of the

pyramidal layer of the cerebral cortex. However, melatonin was able to restore the cytoarchitecture of cells of the pyramidal layer of the cerebral cortex when administered concurrently and after the administration of sodium fluoride (NaF) respectively. Also, melatonin regulated the activities of Superoxide dismutase, Malondialdehyde and Glutathione peroxidase in the cerebrum. Sodium fluoride causes neurodegeneration in the cerebral cortex, and exogenous melatonin can ameliorate the injury caused by sodium fluoride on the cerebral cortex.

Key words: Cerebrum – Neurodegeneration – Cytoarchitecture – Melatonin – Sodium fluoride

INTRODUCTION

Fluoride, a derivative from the element fluorine, is associated to form insoluble complexes with cations like sodium, magnesium, aluminium or calcium (Whitford et al., 1997). These formed complexes have potential roles in biological and toxicological processes (Bigjay et al., 1987). Sodium

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fluoride is an ionic compound formed from sodium ion (Na^+) and fluoride ion (F^-) (ASTDR, 2003; Wells, 1984). Some of the beneficial role of sodium fluoride is that it can be used as both a dietary supplement and multivitamin (Thomson/Micromedex, 2006; McEvoy, 2006). The toxicity effects of fluoride have been reported in various tissues via cell enzyme inhibition and activation, depending on the type of the enzyme involved (Adamek et al., 2005, Mendoza-Schulz et al., 2009). Excessive exposure to fluoride brings about increase in the production of anion superoxide (O_2^-) (Garcia-Montalvo et al., 2009; Hassan and Yousef, 2009) and other hydroxyl radicals that may initiate hazardous effects of fluoride (Urbansky, 2002), ER stress and reactive oxygen species (ROS) production (Hassan and Yousef, 2009; Lui et al., 2003; Sireli and Bulbul, 2004). Fluoride is also known to cross the blood-brain barrier (BBB) to cause neuronal degeneration resulting in central nervous system (CNS) dysfunction (Claro et al., 1990). Myelin splitting, vacuolation of mitochondrial, compressed Golgi cisternae, dilatation, and scattering of the rough endoplasmic reticulum of neurons were all affected after treatment with sodium fluoride on the brain (Reddy et al., 2011). Furthermore, the use of sodium fluoride in the treatment of water and as an additive in toothpaste is still frequent, and it is known to cause a deleterious effect (low intellectual coefficient, neurodegeneration) in the brain (Chauhan et al., 2014). The effect of fluoride on the cerebral cortex of both neonatal and adult rats has been established to show loss of cellular layer and major neurodegeneration changes in the motor cortex (Shivaraishankara et al., 2002; Shashi, 2003).

Melatonin is an indoleamine that is secreted by the pineal gland of the brain to influence the sleep and wake cycle (Choi, 2013), also known as the hormone of darkness (Master-Israilov et al., 2015), and known to have numerous functions such as antioxidant, neuroprotective, anti-inflammatory, anti-apoptotic, or regulatory of energy balance. It is known to freely permeate all morphophysiological barrier of cells in any organ (Shida et al., 1994; Reiter, 1996) and to be concentrated in free-radical generating tissues to prevent potential damage (Reiter, 2000).

Although it has been established that fluoride have the ability to cause deleterious effects ranging from learning and memory deficiency to motor activity impairment (Saad El-Dien et al., 2010; Nasir and Asad, 2013), treatment against this effect has not been established, however. Therefore, it is imperative to understand the possible mechanism of exogenous melatonin against the deleterious effects of fluoride on the cerebrum.

MATERIALS AND METHODS

Chemicals and Drugs

Melatonin: Melatonin in its tablet form was obtained from a local Pharmaceutical Company and produced by Good Neighbour Pharmacy, Broadway industries, United State of America (ABC# 10148547). Melatonin was subsequently dissolved in 0.9 ml of Normal saline (Petri et al., 2011).

Sodium Fluoride: Sodium fluoride salt was obtained from Denis store at Taiwo Road in Ilorin and produced by Guangdong Guanghua Chemical factory co. ltd. Shanton Guangdong, China (#515000). Oxidative stress parameters (MDA), superoxide dismutase (SOD) using SOD assay kit, a product of the Cayman Chemicals, 1180 E. Ellsworth Rd. Ann Arbor, MI. the USA. Item No: 706002, and glutathione GSH using GSH Assay Kit (Colorimetric) Catalog Number KA0797 from Abnova. Sodium fluoride was later administered through drinking water to the animals.

Experimental Design

Forty rats weighing between 150-200g were used for this study with free access to food and water *ad libitum*, and exposed to normal light/dark cycle and normal room temperature/ humidity. Experimental protocols were in strict compliance with the guideline for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (2011) and approved by the ethical committee of the University of Ilorin, Ilorin (UERC/ASN/201/856).

The animals were randomly divided into four groups (I- IV), which received oral administration

of 0.2ml of normal saline (NS), 500ppm of sodium fluoride (Kour and Singh, 1980) (NaF), (MLT), concurrent administration sodium fluoride and melatonin (NaF+MLT), and sodium fluoride before melatonin (NaF-MLT) respectively. Note 10 mg/kg of melatonin was given according to Bustos-Obregón et al., 2013).

Tissue Collection

All antibodies were procured from Dianova (GmbH/Warbugstr. 45/20354 Hamburg. Also, reagents and buffers used in this study were molecular biology grade (99.9% pure) from Sigma-Aldrich. At the end of the various treatments, i.e., twenty-four hours later, the animals were sedated with intramuscular administration of 20 mg/kg of ketamine perfused through the heart (Ajao et al., 2010), and cerebral tissues were excised.

Histological/Histochemical procedures

The excised cerebral tissue was initially fixed in four percent paraformaldehyde overnight after extraction, and later transferred to 30% sucrose solution, before taken for histological and histochemical analyses, which were Hematoxylin and Eosin stain to demonstrate the general cytoarchitecture, and Cresyl Fast Violet stains to demonstrate the presence of Nissl bodies respectively.

Determination of Biochemical Parameters

0.1 g of the cerebrum was extracted from the rest of the brain and homogenized in 0.4 ml of five percent sucrose solution; the tissue was further centrifuged at 3000 rpm for 10 minutes, and the clear supernatant was separated into plain bottles. The supernatant was later taken to determine the level of oxidative stress using Superoxide dismutase, malondialdehyde and Glutathione peroxidase enzymes-linked immunosorbent assay commercial kit (ELISA).

Data Analysis

All data were expressed as mean $\pm$ standard error of the mean. Differences among control and the experimental groups were considered with $P < 0.05$ as statistically significant, using one-way

analysis of variance (ANOVA), followed by Tukey *post hoc* test to determine the differences between the groups. The statistical tests were performed using GraphPad Prism version 5.0.

RESULTS

Qualitative results

Cytological arrangement after exposure to fluoride and melatonin

The control group showed a normal cytoarchitecture pattern with presence of normal granular cells (NGC), and the presence of glial (GC) (Fig. 1), densely stained Nissl substance represented (NC) (Fig. 2). Sodium fluoride (NaF) slide showed presence of damaged cells with shrunken nucleus (Pyknotic cell (PC)), presence of pericellular halos (PH), large-sized granular cells (Fig. 1), and sparsely stained Nissl substance with the presence of vacuolations; pyknotic cells (PC) (Fig. 2). Sodium fluoride and melatonin (NaF+MLT) concurrent group showed cells to have normal cytoarchitecture (NC, NPYC), spindle-shaped pyramidal cell (DPYC), pericellular halos (PH) (Fig. 1) densely stained (NC) and sparsely stained (SC) Nissl substance (Fig. 2); Sodium fluoride-melatonin (NaF-MLT) group showed some normal granular cell (NC, NGC) and shrunken cell (PC) (Fig. 1) and densely stained (NC), sparsely stained (SC) Nissl substance and pyknotic appearance (PC) (Fig. 2).

Quantitative results

The effects of fluoride and melatonin using oxidative stress markers

The activity of SOD in the cerebral cortex of the animals that received sodium fluoride (NaF) group showed a significant decrease compared to the groups that received melatonin as treatment plan; Table 1. In addition, the concentration of the MDA in the cerebral cortex of the control (NS), sodium fluoride+melatonin (NaF+MLT) and sodium fluoride-melatonin (NaF-MLT) animals showed significance decrease as compared to sodium fluoride (NaF) group; Table 1. Also, the activity of glutathione in the cerebral cortex of control (NS), sodium fluoride+melatonin (NaF+MLT), sodium

fluoride-melatonin (NaF-MLT) animals showed statistical significance increase as compared to the sodium fluoride (NaF) group only. Furthermore,

there was no statistically significant difference between NaF+MLT, NaF-MLT groups as compared to control (NS) group Table 1.

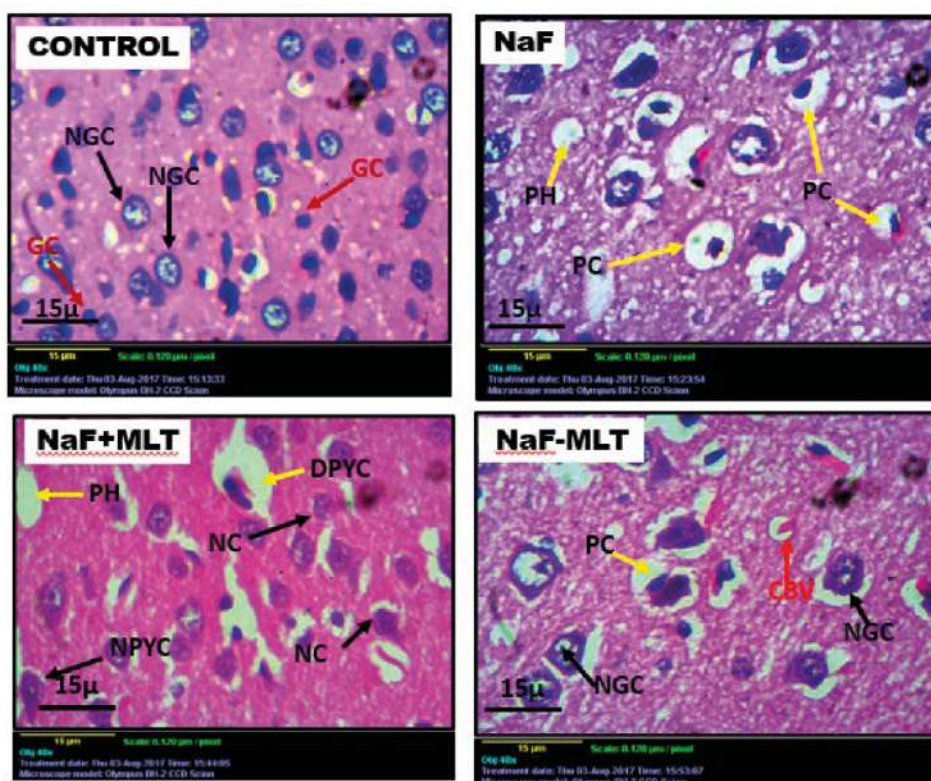


Fig. 1.- Haematoxylin and Eosin stains showing the general cytoarchitecture of the pyramidal layer of the cerebrum of rats. NGC- Normal granular cell; GC- Glia cell; PC- Pyknotic cell; NC- Normal cell; NPYC- Normal pyramidal cell; DPYC- Damaged pyramidal cell; PH- Pericellular halos; PC-CBV- Pyknotic cell with constricted blood vessel; CBV- Constricted blood vessel. Scale bars = 15 µm.

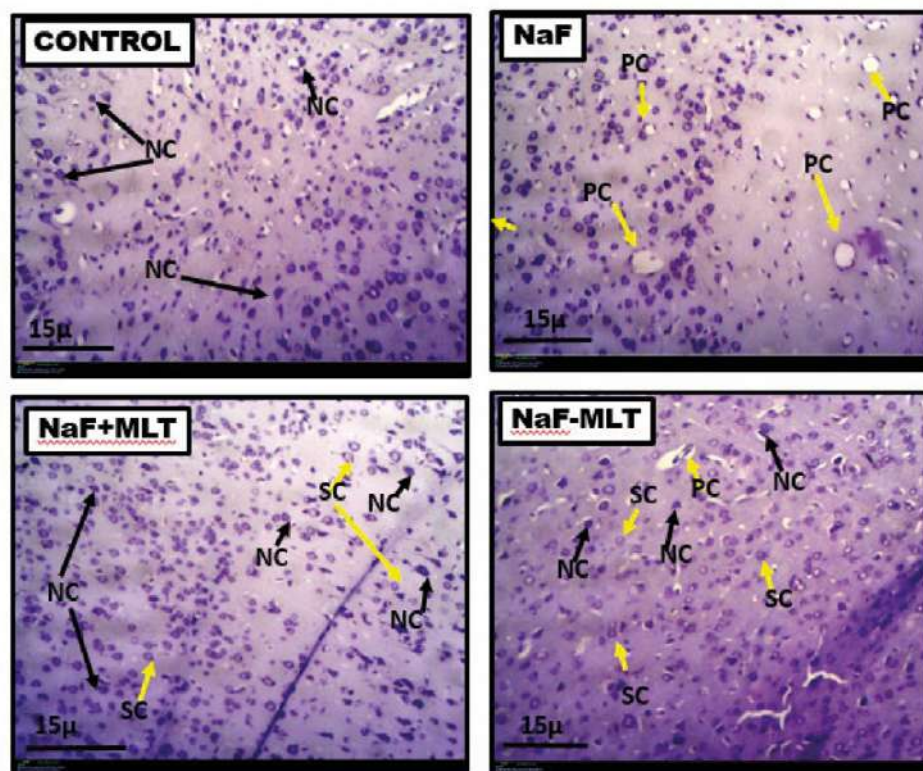


Fig. 2.- Cresyl fast violet stain showing the arrangement of Nissl substance in the pyramidal layer of the cerebrum of rat. NC- Densely stained Nissl substance; PC- Pyknotic cell; SC- Sparsely stained Nissl substance. Scale bars = 15µm.

Table 1. The actions of sodium fluoride and melatonin on oxidative stress markers.

Groups	SOD (U/L) Mean±SEM	MDA (mM) Mean±SEM	GSH (mM) Mean±SEM
NS (control)	2.10±0.10	0.83±0.01	0.14±0.01
NaF	1.19±0.01 ^a	0.99±0.01 ^a	0.08±0.01 ^a
NaF+MLT	1.91±0.01 ^b	0.81±0.01 ^b	0.11±0.01
NaF-MLT	1.61±0.01 ^{ab}	0.84±0.10 ^b	0.13±0.01

^{a,b} statistically significant difference as compared to normal saline (NS), sodium fluoride (NaF) groups respectively ($p < 0.05$).

DISCUSSION

The pyramidal cells in the cerebral cortex showed degenerative changes after administration of sodium, which affected cellular arrangement by inducing the opening of the permeability transition pore and inhibiting the cell membrane potential (Mendoza-Schulz et al., 2009; Chauhan et al., 2014; Anuradha et al., 2001; Geeraerts et al., 1986; Mullenix et al., 1995; Shashi and Kumar, 2016). The ameliorative changes seen were the result of the administration of exogenous melatonin against neural inflammation and apoptotic properties of sodium fluoride neurotoxicity, by acting on some proteins that are involved in the protection of the brain and regulation of its receptors: i.e., melatonin was able to rescue the neural cells through activation of their receptors. (Dun-Xian, 2016; Wang et al., 2009; Tapias et al., 2009; Rao et al., 2010).

The cellular components, the ribosome and endoplasmic reticulum, which are the major sites of protein synthesis in the neuron, were studied by Nissl staining. There was evidence of sparsely stained Nissl bodies due to the loss of the rough endoplasmic reticulum, which is caused by the effect of sodium fluoride. Furthermore, it has been suggested that fluoride degenerates the cell bodies, and this causes the rough endoplasmic reticulum to become scattered (Saad El-Dien et al., 2010; Zhan et al., 2006; Zhang et al., 1999). Also, Reiter suggested that fluoride causes the rough endoplasmic reticulum to become scattered. However, the treatment with melatonin was able to reduce disintegration and dispersal of Nissl bodies, i.e., reducing chromatolysis in these cells by up-regulating the neurotrophic hormone like BDNF, synapsin 1 (Jing et al., 2017).

Sodium fluoride has been established to decrease some enzyme activity in the cells by increasing reactive oxygen species (ROS) in the mitochondria, leading to cellular damage. In this study, the level of superoxide dismutase (SOD) in the group administered sodium fluoride (NaF) and the group which received the treatment before inducing (MLT-NaF) sodium fluoride decreased. However, the treatment with melatonin suggests that melatonin through its antioxidant property was able to increase superoxide dismutase level, which inhibits the production of ROS, thereby inhibiting oxidative stress and cellular damage. These findings also buttress the fact that melatonin increases antioxidant level by mopping/ scavenging of free radicals produced (Reiter, 2000; Zhang et al., 2003; Reiter et al., 2007; Meda et al., 2014; Ajoke et al, 2020).

The level of glutathione was also reduced in the group administered sodium fluoride (NaF), but the administration of melatonin was able to increase the level of glutathione in the cerebral cortex. It can be suggested that melatonin, through its antioxidant property by the importation of cystine for the biosynthesis of glutathione through the cystine glutamate antiporter (system Xc) and exportation of glutamate, leads to inhibition of oxidative stress, which means that melatonin establishes the antioxidant activity through the synthesis and transport of cysteine (Gupta et al., 2003; Clarke et al., 2012; Floreani et al., 1997; Ajoke et al, 2020).

Sodium fluoride can induce lipid peroxidation, which attacks membrane phospholipid and reduces fatty acid concentration. There was a significant increase of malondialdehyde (MDA) in the sodium fluoride (NaF) group as a result of the increase in the production of polyunsaturated fatty

acid, similarly to the treatment before induction (MLT-NaF) group in the cerebral cortex of Wistar rats. However, the treatment group that received melatonin showed decrease in lipid peroxidation. This finding suggests that melatonin can regulate the concentration of malondialdehyde in the cerebral cortex of Wistar rats by inhibiting lipid peroxidation cascade/ pathway; which adds to the fact that melatonin is able to restore fatty acid concentration by decreasing lipid peroxidation (Meda et al., 2014; Baydas et al., 2002; Rodriguez et al., 2004).

SUMMARY OF FINDINGS

Melatonin reduced the rate of neural inflammation and also regulated the process of apoptosis in the cells of a damaged cerebrum (Fig. 3). Furthermore, melatonin promoted protein synthesis by reducing chromatolysis in the cells of the cerebrum (Fig. 3). Lastly, melatonin limited the extent of oxidative stress by increasing the levels of superoxide dismutase, increased the importation of cysteine for the biosynthesis of glutathione, and reduced the amount of oxidative degradation of lipids in the cerebrum (Fig. 3).

CONCLUSION

At the end of the study, melatonin was able to limit the extent of sodium fluoride damage by causing reduction in neural inflammation and regulating apoptosis, reduction in the proliferation of chromatolytic cells and reduction in the generation of free radicals. Therefore, melatonin (exogenous) acts as an ameliorative substance on the cyto-architectural and biochemical damage induced by sodium fluoride on the cerebrum of adult Wistar rats.

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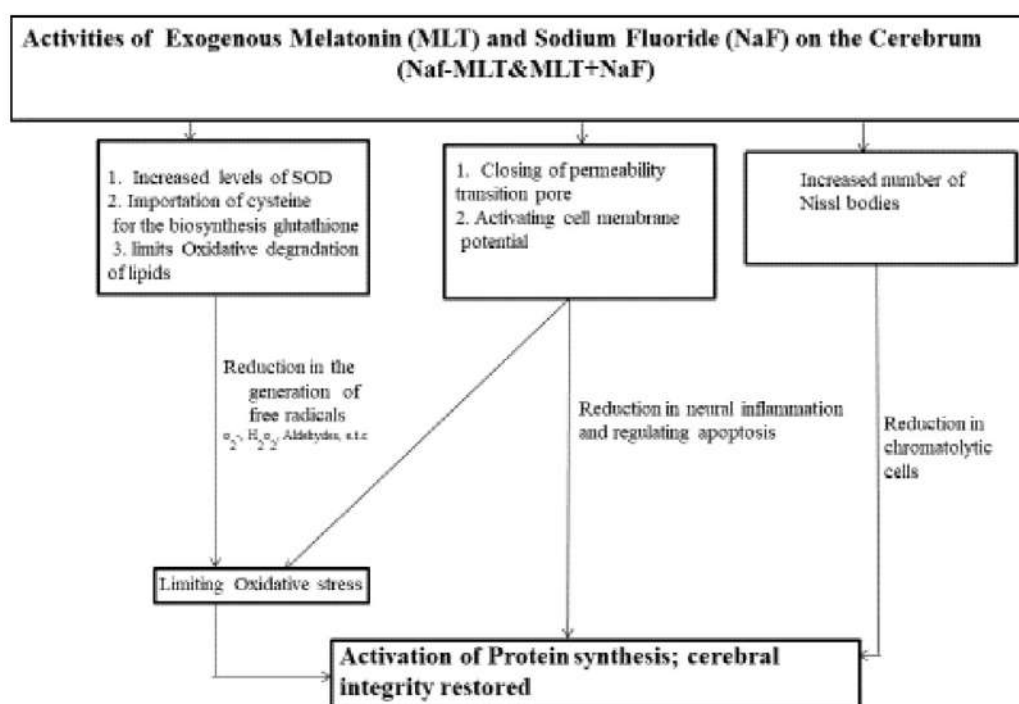


Fig. 3.- Role of exogenous melatonin on sodium fluoride induced cerebellar damage.

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