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Research Article
Neurological Effects of Cobalt Chloride on the Cerebral Cortex of Adult Wistar Rats

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Abstract

Background and Objective: Cobalt is a naturally-occurring trace element with a wide range of industrial and medical applications. In daily life, humans are exposed to Cobalt through inhalation, drinking water and food. The study investigated the effects of cobalt chloride on the cerebral cortex in Wistar rats. **Materials and Methods:** Thirty-Six healthy adult Wistar rats of both sexes weighing 170-220 g were separated into four groups, A, B, C and D with each group containing nine animals. Group A rats were the control, while group B, C and D were treated orally with Cobalt chloride 66.8, 145 and 223.2 mg kg^{-1} body weight for 23 days, respectively. The rats were sacrificed and the brain was removed and weighed using a sensitive weighing balance, part of the brain was homogenized for biochemical analysis for MDA. The SDH and NO, while the remaining part was fixed in 10% formol calcium and processed for histological study. **Results:** The mean body weights of the Wistar rats decreased significantly ($p<0.05$) in groups B, C and D treated groups compared with Group A. The brain weights in groups B, C and D treated groups also decreased insignificantly ($p>0.05$) when compared with group A biochemical analysis indicated a significant increase ($p<0.05$) in the level of MDA while SDH and NO levels were significantly reduced ($p<0.05$) in group B, C and D treated groups as compared with group A. Histological study of the cerebral cortex revealed that the cerebral cortical layers in groups C and D appeared distorted and degenerated in a dose-dependent manner as compared with group A which shows a normal cerebral cortical architecture. **Conclusion:** The cobalt chloride administration has a neurotoxic effect on the cerebral cortical layers in adult Wistar rats which may adversely affect some cerebral cortical functions.

Key words: Neurotoxic, cerebral cortex, cobalt chloride, neurodegeneration, oxidative stress, neurons

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The cerebrum directs the conscious or volitional motor functions of the body. These functions originate within the primary motor cortex and other frontal lobe of motor areas where actions are planned. Upper motor neurons in the primary motor cortex send their axons to the brainstem and spinal cord to synapse on the lower motor 24 neurons, which innervate the muscles. The neurons of the cerebral cortex are grouped into six main layers, from outside to inside and damage to motor areas of cortex can lead to certain types of motor neuron disease[1].

The cerebral cortex, also referred to as the cerebral mantle is the outer cortical layer of neural tissue of the brain in biological systems. The cerebral cortex primarily is described as having six- cortical layers of neocortex essentially while the allocortex consists of only 10%. The cerebral cortex performs a crucial role in language, consciousness, perception, awareness, thought and memory. The cortex has two cortices with a longitudinal fissure separating the two parts of the cerebrum into the left and right cerebral hemispheres. The cerebral cortex has been shown to possess the largest site of cortical neural integration in the central nervous system of the brain[1].

Reports from previous studies have indicated that in most mammals, apart from small mammals with small brains, the cerebral cortex is folded, providing a greater surface area in the confined volume of the cranium, cortical folding is crucial for the wiring of the brain and its functional organization in addition to minimizing brain and cranial volume. However, in mammals with a small brain, there is no folding and the cortex is smooth[2].

Report from previous studies has implicated cobalt chloride as a cytotoxic substance to numerous tissues, cell types, including neural cells coupled with increased production of reactive oxygen species (ROS) and DNA fragmentation which also induces cell death through the mechanism of necrosis and apoptosis in tissues[3]. Findings from previous studied have indicated cobalt to be an environmental toxicant and excessive bodily exposure may predispose to damage of nervous system resulting in cobalt-induced neurodegeneration occasion by neurotoxicity in tissues[4].

Cobalt is a naturally occurring element in the earth's crust that is a component of vitamin B12, which supports the production of red blood cells. Very small amounts are also required in animals and humans to stay healthy. Previous investigators have revealed that cobalt poisoning may occur following exposure to large amounts of the substance. Three possible ways have been outlined through which cobalt can cause poisoning, swallowing the substance, inhalation through the nose into the lungs and repeated contact with skin[5]. Moreover, a previous study has implicated the involvement of cobalt particles resulting in cobalt-induced toxicity in blood and tissues when the ball exhibits grinding action against the metal cup while walking in humans[6].

Studies from previous investigations have shown that increased cobalt levels may be associated with a variety of neurological disorders in addition to "classic" and known cardiac diseases such as arrhythmias and cardiomyopathies as well as allergic or endocrine symptoms[7]. Reports from the literature have revealed that heavy metals including cobalt chloride have become issue of concern and increasingly implicated in the multi-factorial aetiology of several neurological disorders as cobalt is known to be a potent hypoxia-inducing agent, acting through some mechanisms in association with hypoxia-inducible factor leading to cell injury and apoptosis[8].

Some studies have indicated that cobalt intoxication induces depletion of serotonin, dopamine and norepinephrine, as well as distortion in the retina and the optic nerve. Cobalt is known to induce loss of mitochondrial membrane potential and the release of apoptogenic factors which is involved in the pathogenesis of cobalt optic neuropathy, the alteration of mitochondrial oxidative phosphorylation plays a fundamental role in these conditions. Findings from histopathological studies have demonstrated substantial cerebral and cerebellar damage with characteristic features of neuronal necrosis when compared with controls that appeared normal. In the cerebral cortex, the neurons, glial cells and neuropil were adversely altered and the cerebellar Purkinje cells appeared degenerated which ultimately indicated the neurotoxic potential of cobalt chloride and the associated health risks in newborns and infants, particularly in areas with cobalt exposure and pollution[9].

Moreover, it has been observed that neurotoxicity occurs following chronic exposures to cobalt chloride, although there is a paucity of information in the literate concerning the mechanism involved in cobalt-induced neurotoxicity. The evidence and implications of cobalt chloride toxicity on some body functions have been well reported by previous investigators in association with occupational chronic exposures to cobalt chloride as well as with the medical treatment of some clinical conditions.

Furthermore, findings from other studies have indicated that Individuals that presented significant levels of exposure to cobalt chloride released through metallic prosthesis have been observed to have various neurological disorders including visual atrophy, tremor, vertigo, peripheral neuropathy and deafness during clinical examination[10]. In view of the previous reports in association with the neurotoxicity of cobalt chloride, this study appraised the neurohistological and biochemical changes in the cerebral cortex in Wistar rats following cobalt chloride exposures.

MATERIALS AND METHODS

The study was carried out between June, 2021 and February, 2022 at the Animal House of the Anatomy Department, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, served as the location for this study.

Experimental animals: Thirty-six healthy adult Wistar rats of both sexes were obtained from the Animal House of the Department of Human Anatomy, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Ogbomoso. The adult Wistar rats were fed with rat feed and received distilled water *ad libitum* and were also kept and maintained in the departmental animal house for two weeks to acclimatize prior to the study. The adult Wistar rats of both sexes weighed 170-220 g. The Wistar rats were treated by following the, "Guide for the Care and Use of Laboratory Animals" compiled by the National Academy of Science and Published by the National Institute of Health[11].

The body weights of the experimental animals were monitored and weighed with a sensitive weighing scale and the values were recorded. The body weights were taken during acclimatization periods and during administration to monitor and study the effect of the administration of cobalt chloride on the body weight of the adult Wistar rats.

Experimental design and grouping: Thirty-six adult Wistar rats of both sexes weighing 170-220 g were separated into four groups based on their body weight range:

- **Group A (Control group):** Received distilled water for 23 days
- **Group B:** Received 66.8 mg kg^{-1} of cobalt chloride orally for 23days
- **Group C:** Received 145 mg kg^{-1} of cobalt chloride orally for 23 days
- **Group D:** Received 223.2 mg kg^{-1} of cobalt chloride orally for 23 days

Cobalt chloride administration to the rats was done using the oral route of administration.

The animals were sacrificed on the 24th day of the treatment by cervical dislocation which temporarily rendered the animal unconscious. The brain of each rat was carefully harvested and weighed with a sensitive weighing balance, part of it was homogenized for biochemical analysis (SDH, MDA and NO), while the remaining part was then fixed in 10% formol calcium for morphological analysis using H and E staining techniques.

Ethical consideration

Ethical approval: All authors hereby declare that the principles of laboratory animal care [NIH publication No. 85-23 revised 1985] were followed as well as specific national laws where applicable. All experiments have been examined and approved by the relevant ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and therefore have been performed by the ethical standards laid down in the 1964 Declaration of Helsinki.

Statistical analysis: All data were expressed as Mean±SEM. The statistical analysis of the results obtained in this study was evaluated and tested for significance using Student's t-test. If p-value of the t-test was less than 0.05 ($p<0.05$), then the result was significant. If p-value of the test-test was greater than 0.05 ($p>0.05$), then that means that the result was not significant.

RESULTS

The body weights of Wistar rats in the control group and treatment groups were shown in Table 1. A significant reduction ($p<0.05$) in the initial and final weights in groups B, C and D as compared with group A were shown in Table 2. An ($P>0.05$) decrease in the brain weight in Group B, C and D as compared with Group A was shown in Table 3. An increase in the level of MDA in the treated groups when compared with the control, increased significantly ($p<0.05$) from 3.270±0.55 to 8.690±2.2 in group B, 14.77±0.95 in group C and 18.02±2.09 in group D as shown in Table 4.

Histological observation: Representative micrographs of H&E staining showing the general and magnified cytoarchitecture of the cortex (brain) in male Wistar rats (X100 and X400, respectively). Normal histological features of the cortex were seen in groups A and B (Fig. 1 and 2) treatment is characterized by large pyramidal as well as granule neurons the pyramidal cells are characterized by long

Table 1: Mean±SEM of the body weights of Wistar rats before and during the administration

Period/Week	Group A	Group B	Group C	Group D
Week 0	246.7±21.34	194.4±6.04*	181.1±4.85**	198.8±1.25
Week 1	248.9±18.89	197.8±9.69*	192.5±9.21*	210.0±8.55
Week 2	275.0±20.27	197.5±9.59**	197.1±6.88**	208.0±8.00*
Week 3	262.5±21.86	177.5±7.01**	180.0±6.17**	193.3±4.22*

Significance: p<0.05, a value greater than 0.05 were considered insignificant while values less than 0.05 are considered significant (*). Values were expressed as Mean±Standard Error of the Mean

Table 2: Mean±SEM of the body weights of Wistar rats before and after cobalt chloride administration

Groups	Initial weight	Final weight	Weight gain/loss (%)
A	246.7±21.34	262.5±21.86	6.40
B	194.4±6.04	177.5±7.01**	-8.69
C	181.1±4.85	180.0±6.17**	-6.07
D	198.8±1.25	193.3±4.22*	-2.77

Significance: p<0.05, values greater than 0.05 were considered insignificant while values less than 0.05 are considered significant (*). Values were expressed as Mean±Standard Error of the Mean

Table 3: Mean±SEM of BRAIN weights of adult Wistar rats after cobalt chloride administration

Groups	Brain weight	Relative weight of brain (%)
A	1.686±0.05	0.64
B	1.338±0.12*	0.75
C	1.466±0.06*	0.81
D o/87	1.620±0.07	0.84

Significance: p<0.05, a value greater than 0.05 were considered insignificant while values less than 0.05 are considered significant (*). Values were expressed as Mean±Standard Error of the Mean

Table 4: Effects of cobalt chloride on Mean±SEM of MDA, SDH and NO

Groups	No	MDA	SDH
A	15.39±1.46	3.270±0.55	3.895±0.35
B	8.998±1.45*	8.690±2.2*	2.415±0.57
C	9.138±0.57**	14.770±0.95***	1.660±0.31***
D	8.292±0.61**	18.02±2.09***	1.412±0.22***

Significance: p>0.05, a value greater than 0.05 were considered insignificant while values less than 0.05 were considered significant (*). Values were expressed as Mean±Standard Error of the Mean. NO: Nitric oxide, MDA: Malondialdehyde and SDH: Succinate dehydrogenase

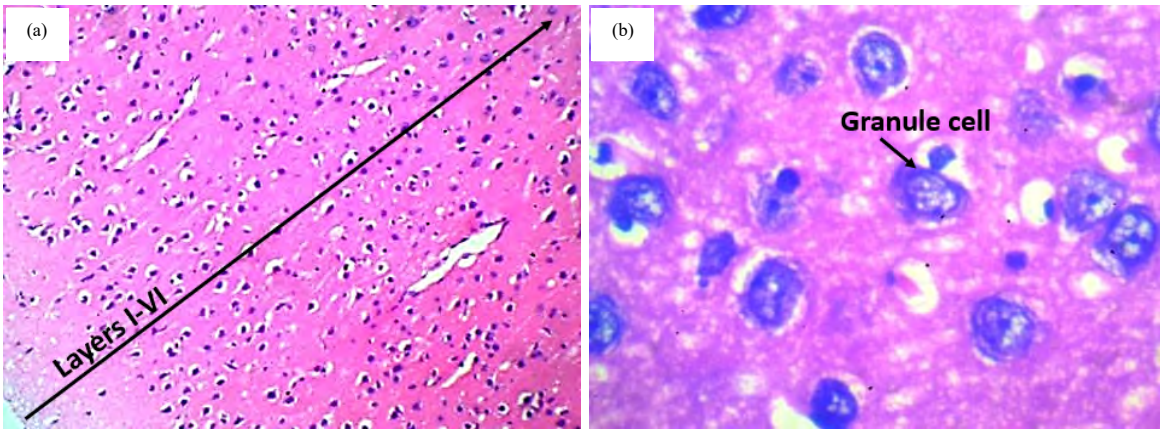

Fig. 1(a-b): Group A: Control group

A: Photomicrographs showing a normal histological feature of the cerebral cortex, characterized by large pyramidal cell, with long axons that extends well from the delineated soma of the pyramidal neurons, normal molecular layers and external granular layer also appear normal (H&E ×100 ×400)

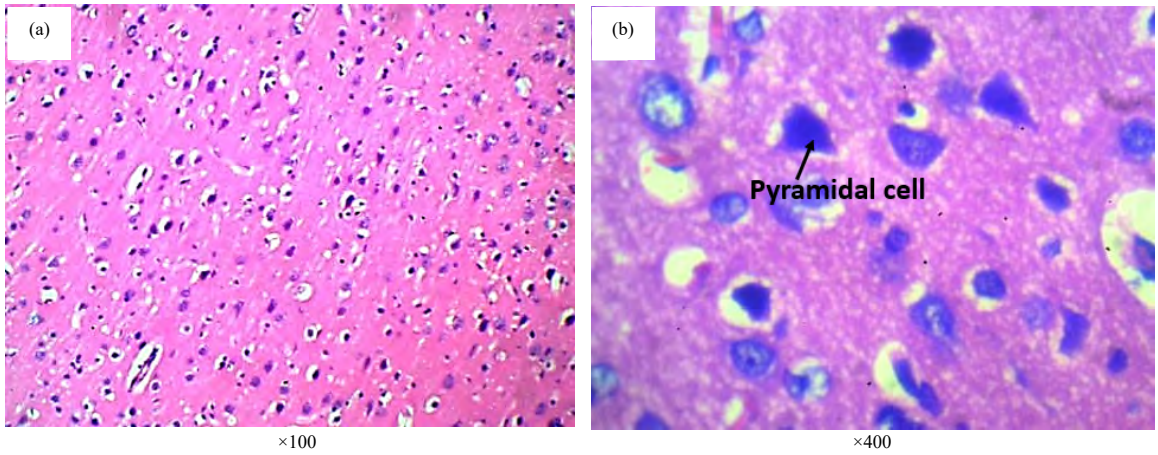

Fig. 2(a-b): Group B: Treated with 66.8 mg kg^{-1} of cobalt chloride for 23 days

Photomicrograph showing the treatment of group B is characterized by large pyramidal as well as granule neurons the pyramidal cells are characterized by long axons that extend well from the soma to adjacent neurons within the neuropil. Apical and basal dendrites extend from the well-delineated soma of the pyramidal neurons in this group. Perineural space surrounding these cells appears intact, with intact nuclear and cytoplasmic content

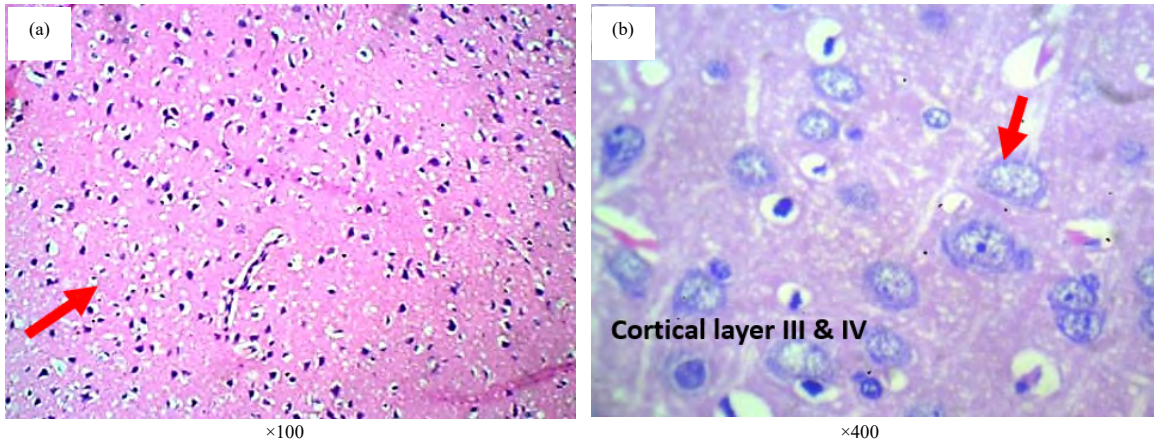

Fig. 3(a-b): Group C: Treated with 145 mg kg^{-1} of cobalt chloride for 23 days

Photomicrograph of group C treatment shows mild conspicuous degenerative changes in the brain that were characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within soma (red arrow). Perineural spaces can be seen surrounding degenerating neurons, Axons and dendrites are scarcely appreciable around neurons in this group and the neuronal population appears scarcely appreciable in this group

axons that extend well from the soma to adjacent neurons within the neuropil. Apical and basal dendrites extend from the well-delineated soma of the pyramidal neurons in this group. Perineural space surrounding these cells appears intact, with intact nuclear and cytoplasmic content. Group C and D (Fig. 3 and 4) treatment caused mild to severe conspicuous degenerative changes in the brain that was characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within soma (red arrow). Perineural spaces can be seen surrounding degenerating neurons, axons and dendrites are scarcely appreciable around neurons in this group and the neuronal population appears scarcely appreciable in this group (Fig. 3 and 4).

Photomicrographs showing panoramic views of the cortex (brain) general micromorphological presentations in male Wistar rats across the study groups. The H&E stain (X100 magnification). The (I) Molecular layer, (II) External granular layer, (III) External pyramidal layer, (IV) Internal granular layer, (V) Internal pyramidal layer and (VI) Multiform layer are demonstrated across study groups (white line).

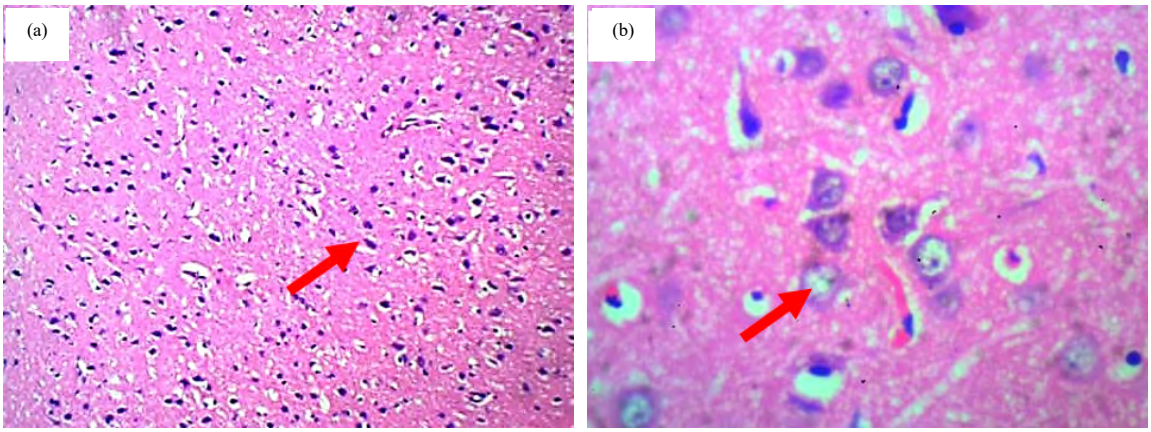

Fig. 4(a-b): Group D: Treated with 223.2 mg kg^{-1} of cobalt chloride for 23 days

Photomicrograph of group D treatment shows severe conspicuous degenerative changes in the brain that were characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within soma (red arrow). Perineural spaces can be seen surrounding degenerating neurons, Axons and dendrites are scarcely appreciable around neurons in this group and neuronal populations appear scarcely appreciable in this group

DISCUSSION

This study investigated the histomorphological effects of cobalt chloride on the cerebral cortex of adult Wistar rats. The study investigated the effect of cobalt chloride administration on adult Wistar rat brains and the effect of cobalt chloride on the body weight, mean and relative organ weight in adult Wistar rats. Cobalt is a natural element found throughout the environment. Cobalt chloride is an essential trace element being an integral part of vitamin B12. The administration of cobalt chloride to experimental rats has been shown to cause an insignificant decrease ($p>0.05$) in group B (cobalt chloride-treated group) which was exposed to 66.8 mg kg^{-1} of cobalt chloride when compared to group A (control group). The body weight of animals in group C which was exposed to 145 mg kg^{-1} of cobalt chloride decreased significantly ($p<0.05$) when compared to the body weights of animals in group A (control group). The body weight of animals in group D which was exposed to 232.2 mg kg^{-1} of cobalt chloride decreased significantly ($p<0.05$) when compared to the body weights of animals in group A control group). This possibly implied that cobalt chloride decreases feed intake which resulted in a significant decrease in the weight of animals in group B, group C and group D compared to group A, which was previously revealed by previous investigators who observed a significant decrease in cobalt chloride treated group in their studies[12].

Comparing the relative brain weight of group B (cobalt chloride-treated group) which was exposed to 66.8 mg kg^{-1} of cobalt chloride, a significant increase ($p<0.05$) was observed when compared to the relative brain weight of group A (control group). In the relative brain weight of group C which was exposed to 145 mg kg^{-1} of cobalt chloride, shows a significant increase ($p<0.05$) was observed when compared to the relative brain weight of group A (control group). For the relative brain weight of group D which was exposed to 232.2 mg kg^{-1} of cobalt chloride, shows a significant increase ($p<0.05$) was observed when compared to the relative brain weight of group A (control group) This result shows a decrease in the brain weights of group B, group C treated with cobalt chloride an observation that was similar to the findings of the previous investigators that cobalt chloride affected the brain thereby damaging and decreasing the weight.

The results of the biochemical parameters investigated show a significant increase ($p<0.05$) in the level of MDA (malondialdehyde) in groups B, C and D received 66.8, 145 and 232.2 mg kg^{-1} of cobalt chloride, respectively as compared with group A and these findings were supported by a study previously carried out by the previous investigators[13]. The level of NO (nitric oxide) in groups B, C and D received 66.8, 145 and 232.2 mg kg^{-1} of cobalt chloride also decreased significantly ($p<0.05$) as compared with group A. The level of SDH (Succinate dehydrogenase) in groups B, C and D received 66.8, 145, and 232.2 mg kg^{-1} of cobalt chloride reduced significantly ($p<0.05$) as compared with group A. It has been shown that MDA and NO are an indicator of oxidative stress while SDH is a marker of mitochondria enzymes which builds up an electrochemical gradient across the mitochondrial inner membrane allowing for the synthesis of ATP. Alternatively, electrons can be diverted to reduce the ubiquinone pool (UQ pool) and provide reducing equivalents necessary to

reduce superoxide anions originating either from an exogenous source or from the respiratory chain itself. However, the results of this biochemical assay were in agreement with the previous work done[14].

Malondialdehyde (MDA) is a compound that describes the activity of free radicals in cells so it is used as one of the indications of oxidative stress caused by free radicals Another study reinforces this statement by stating that the mediator malondialdehyde (MDA) is a final product of fat peroxidation which was used as a biological biomarker of fat peroxidation and can describe the degree of oxidative stress[15].

The MDA, NO and SDH levels have a strong significant correlation and have a reciprocal relationship which means that the higher the MDA level the lower the level of NO and SDH in the body. This was consistent with another study which indicated that there was an increase in MDA levels and a decrease in succinate dehydrogenase levels in cement workers[16].

The histological observation in group A shows a normal histological feature of the cerebral cortex, characterized by a large pyramidal cell, with long axons that extends well from the delineated soma of the pyramidal neurons, normal molecular layers and the external granular layer also appears normal (Fig. 1). The histological section of group B (treated with 66.8 mg kg^{-1} of cobalt chloride) is characterized by large pyramidal as well as granule neurons the pyramidal cells are characterized by long axons that extend well from the soma to adjacent neurons within the neuropil. Apical and basal dendrites extend from the well-delineated soma of the pyramidal neurons in this group. Perineural space surrounding these cells appears intact, with intact nuclear and cytoplasmic content (Fig. 2). The histological section of group C (treated with 145 mg kg^{-1} of cobalt chloride) treatment shows mild conspicuous degenerative changes in the brain that were characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal neurons that appear with fragmented cytoplasm and condensed nuclei within soma (red arrow). Perineural spaces can be seen surrounding degenerating neurons, Axons and dendrites are scarcely appreciable around neurons in group C and the neuronal population appears scarcely observable in group C (Fig. 3). The histological section of group D (treated with 232.2 mg kg^{-1} of cobalt chloride) shows severe conspicuous degenerative changes in the brain that were characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal neurons that appeared with fragmented cytoplasm and condensed nuclei within soma (red arrow). Perineural spaces were seen surrounding degenerating neurons, Axons and dendrites are scarcely observed around neurons in group D and the neuronal population appears scarcely in group D (Fig. 4). This observation was similar to a previously reported study that indicated that graded doses of cobalt chloride caused neurotoxicity and altered the histomorphological architecture of hippocampo-amygdala section of the brain of adult Wistar rats by triggering neurodegeneration[17]. In view of the neurodegenerative changes and oxidative damage of the cortical layers of the cerebral cortex following cobalt chloride exposure in Wistar rats. Further studies will provide information in relation to the mechanism of cobalt chloride-induced-immunohistochemical expression to corroborate the findings from this study in Wistar rats.

CONCLUSION

This study concluded that exposure to high doses of cobalt chloride has adversely affected the cerebral cortical layers which could be a risk factor inducing cellular damage and neurodegeneration in diseases that may adversely impair cerebral cortical functions in adult Wistar rats investigated.

SIGNIFICANCE STATEMENT

This work was done to elucidate the neurotoxic effect of cobalt chloride on the cerebral cortex of Wistar rats using morphological and oxidative stress indices to assess the possible induced damage following cobalt chloride exposure. The findings from the study indicated oxidative and neurodegenerative damage to the cortical layers with degenerating and loss of pyramidal neurons coupled with significantly increased oxidative stress parameters which invariably could result in neurological disorders and compromise of cerebral motor functions.

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