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AMLODIPINE (BESYLATE) EFFECTS ON MEMORY, PSYCHOMOTOR ACTIVITIES AND SOME BIOCHEMICAL MARKERS ON CADMIUM-INDUCED BRAIN TOXICITY IN WISTAR RATS

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ABSTRACT

In the present investigation, Wistar rats subjected to cadmium toxicity were used to examine the effects of amlodipine on memory, psychomotor activities, and some biochemical markers. In this study, 24 Wistar rats with average weight of 115g were separated into 4 groups. Group 1 served as the control group and received regular animal feed and water as needed. In the first phase of the study, all the experimental groups had daily oral administration of 25mg/kg of cadmium for 3 weeks prior to the administration of amlodipine. While in the second phase, Group 2 received 3 mg/kg of amlodipine, Group 3 received 5 mg/kg, and Group 4 received 7 mg/kg alongside the continuous administration of cadmium. The administration of test drugs lasted for 4 weeks, and neurobehavioural tests, including the Barnes Maze, Navigation Maze, and Passive Avoidance tests, were conducted on a weekly basis. After 4 weeks of treatment, animals were sacrificed, and brain tissues were homogenized for acetylcholinesterase, levels of antioxidants, & nitric oxide assays using Elisa Kits. It was found that cadmium was harmful to memory and psychomotor function. While there were no substantial modifications in nitric oxide, Cadmium significantly down-regulate total antioxidant capacity, and increased activities of acetylcholinesterase. However, the effects of Cadmium was ameliorated with amlodipine administration.

KEYWORDS: Cadmium toxicity, Amlodipine, Psychomotor activities, memory, acetylcholinesterase, antioxidant.

1. INTRODUCTION

Cadmium occurs as a minor component in most zinc ores and is a by-product of zinc production. Cadmium was used for a long time as a corrosion-resistant plating on steel, and cadmium compounds are used as red, orange and yellow pigments, to color glass, and to stabilize plastic. Cadmium use is generally decreasing because it is toxic (it is specifically listed in the European Restriction of Hazardous Substances Directive[1,2,3] and nickel-cadmium batteries have been replaced with nickel-metal hydride and lithium-ion batteries. One of its few new uses is in cadmium telluride solar panels.

Although cadmium has no known biological function in higher organisms, a cadmium-dependent carbonic anhydrase has been found in marine diatoms.[4]

Following absorption by either lung or the intestinal epithelium, Cd enters the systemic circulation. Blood Cd concentration serves as a biomarker for Cd exposure level and some data indicated that blood Cd concentration in exposed individuals may range from just above 0 μM to 0.05 μM (REF). Nonetheless, the blood Cd concentration of human varies remarkably according to age, gender, diet, residential area, and smoking status.[5] The effect of Cd exposure is strictly dose-dependent: at high doses Cd can progressively elicit cell injury, cell death, and organ failure, while at low doses, it may modulate specific mechanisms without marked cellular toxicity.[6]

From the outside of cells, Cd can alter the intracellular concentration of calcium which is a universal and versatile intracellular signal messenger.[7] Inside cells, Cd regulates Ca2+ signaling by exerting opposite effects on internal Ca2+ pools. It blocks the release of stored Ca2+ by inhibiting the activity of 1, 4, 5-trisphosphate (IP3) and ryanodine receptors. In contrast, it increases intracellular Ca2+ concentration by promoting calcium efflux from the sarcoplasmic reticulum.[8] Cd can penetrate into neurons via voltage-gated calcium channels.[9] Indeed, a large body of *in vivo* and *in vitro* studies showed that exposure to Cd significantly affects the function of the peripheral (PNS)[10] and central nervous system (CNS)[11] with a wide spectrum of clinical symptoms including olfactory dysfunction, peripheral neuropathy, neurological disturbances, mental retardation, and learning disabilities, as well as motor activity impairment and behavioral alterations both in adults and in children.[12] Moreover, Cd-dependent neurotoxicity has been linked also related to neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases (PD)[13], as well as amyotrophic lateral sclerosis and multiple sclerosis[14], and myalgic

encephalomyelitis.[15] The potential health effects of Cd on humans have attracted a lot of attention over the years, since studies showed that Cd accumulates in tissues and has a very long biological half-life. In humans and other mammals, acute Cd intoxication leads to the development of lesions in a number of organs and tissues, such as the liver, kidneys, lung, pancreas, testes, and bones.[16] In the brain, the accumulation of Cd causes very serious toxic effects[17], and leads to the development of several neurological disorders in workers exposed to Cd, such as hyperactivity, memory loss, and learning difficulties.[18]

Amlodipine is in a class of medications called calcium channel blockers. It lowers blood pressure by relaxing the blood vessels so the heart does not have to pump as hard. It controls chest pain by increasing the supply of blood to the heart[19], Calcium channel blockers are classified as dihydropyridines or nondihydropyridines depending on their chemical structure. Amlodipine has a dihydropyridine ring as part of its structure and is used both for its antihypertensive and anti-angina properties. Several studies have sought to determine the efficacy of amlodipine in comparison to other commonly used antihypertensive including two large studies with pre-defined subpopulations with diabetes.[20] Amlodipine works by blocking the voltage-dependent L-type calcium channels, thereby inhibiting the initial influx of calcium. Reduced intracellular calcium leads to decreased vascular smooth muscle contractility, increased smooth muscle relaxation, and resultant vasodilation. Additionally, amlodipine has been shown to improve vascular endothelial function in hypertensive patients. In summary, amlodipine decreases blood pressure by inducing smooth muscle relaxation and vasodilatation.[21]

Following several scientific publications on the toxic effects of cadmium on memory, psychomotor activities and biochemical markers, the current study was aimed at investigating the possibility ameliorating effects of amlodipine. The specific objectives of the study was to investigate the effect of amlodipine on cadmium-induced changes on memory, psychomotor activities, brain total antioxidant status, brain acetylcholinesterase (AChE), Nitric oxide.

2. MATERIALS AND METHODS

A total of twenty-four male Wistar rats weighting 110-120g were purchased from the experimental animal unit of Department of Human physiology, University of Port Harcourt, Rivers State. They were housed in a disinfected wooden cage, at a room temperature of 22^0C in a 12 hours light: 12 hours dark cycle, and were allowed to acclimatize to the new environment for two weeks, with access to clean water and animal feed provided ad libitum.

A total of twenty-four male Wistar Rats were divided into four groups according to their body weight and grouped according to the experimental design as follows:
Group 1 animals (control) were given only clean water and feed, no frequent was administered to the control animals. This study utilized 3 experimental groups (group II, III, IV). The experimental groups Group II, III & IV received cadmium salt (CAD salt), orally at a dose of 25mg/kg + feed + water daily for 3 weeks (28 days) within these period spatial learning and memory test was conducted on day 7, 14, 21, and 28 to study the progression of cognitive decline and learning deficits in the rats.

At the end of day 28, the first sacrifice was done, and blood sample taken to biochemistry laboratory of the department of biochemistry, University of Port Harcourt for Assay of cadmium – induced changes. At this point, treatment with Amlodipine drug commenced immediately, amlodipine drug was administered orally at varying doses daily to Group II, III & IV for 3 weeks, for possible reversal of the cadmium-Induced changes. At the end of the treatment phase (21 days), the final sacrifice was done, Brain tissues homogenates were taken to the laboratory of University of Port Harcourt and assay for cadmium + Amlodipine induced changes in brain acetylcholinesterase (AchE), brain total antioxidant status and Brain Nitric Oxide.

Experimental protocol for Amlodipine Drug Treatment

Amlodipine is a calcium – channel blocker (calcium antagonist); it is an anti-angina agent, it works by relaxing blood vessels, so blood can flow more. Amlodipine drug treated rats are Group II, III & IV, Amlodipine drug was administered orally daily at different doses.

Group II was administered with Amlodipine drug at 3mg/kg (low dose), Group III received Amlodipine drug at 5mg/kg (medium dose) and Group IV received Amlodipine drug at 7mg/kg (high dose). During these treatment phases of the study, cadmium salt was also administered at a dose of 25mg/kg – the cadmium salt was first administered and then waited for 10 minutes before administering the Amlodipine drug. The procedure was repeated for all the Groups (Group II, III & IV).

Passive avoidance test procedure

It is a fear-aggravated test used to evaluate learning and memory in rats. Models of CNS disorder. Passive avoidance test is an associative learning task memory.[22] The passive avoidance box has two (2) compartment separated by a sliding door, the first compartment is

the brightly lit compartment and the second compartment is dark compartment. During this test the rats learn to suppress a motor response to avoid exposure to the test area (context) associated with or predictive of aversive events such as the dark compartment of the passive avoidance box.[23]

Naturally, rate inhabits in dark places, if during the test there's progressive cognitive decline or memory impairment in the rat, after running out of the dark compartment to escape the foot shock and fear, after some time the rat returns back to the dark compartment.

Barnes Maze Test Procedure

Based on the Delayed Match-to-Place experiment conducted in a standard water maze tank, the Modified Barnes Maze test assesses cognitive deficits in rodent models of CNS disorders.

The Barnes maze is an apparatus utilized in mental research facility examinations to gauge spatial learning and memory. The test was first evolved by Dr. Carol Barnes in 1979.[24]

Principles of Barnes test

The Modified Barnes Maze is thought to measure similar learning abilities as the DMP without forcing the subjects to perform a task under unnatural conditions, i.e. swimming in water. Testing occurs on a circular platform with numerous escape holes ringed around the center of the platform. Bright overhead lighting creates an aversive stimulus, encouraging the animal to seek out the Target Escape Hole, which is attached to an escape tube, and escape from the light.[25] Visual cues placed around the maze act as spatial cues.

Data across the groups with different concentrations of the treatment agents were analysed using one way analysis of variance (ANOVA). Thereafter, the post-hoc test of multiple comparisons (Newman Keuls test) was used to test the individual groups against each other. Confidence level was set at 95% and P-value <0.05 was considered significant.

Ethical Approval

An approval for this study was obtained from the Centre for Research Ethics and Management of the University of Port Harcourt**.**

3. RESULTS AND ANALYSIS

Table 1: Result of Navigation Maze following treatment with various doses of Amlodipine.

GROUPS	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GRP 1 Control	73.66±13.16	167.77±32.22	114.77±45.08	178.83±11.27	171.33±28.66
GRP 2 25mg/kg (cadmium) + 3mg/kg(Amlodipine)	285.11*±14.88	271.88*^{r}±28.11	278.00*^{r} ±0.00	100.00*^{r} ±0.00	100.00*^{r} ±0.00
GRP 3 25mg/kg (cadmium) + 5mg/kg (Amlodipine)	271.38*±28.61	280.00*±0.00	240.38*^{r}±59.61	163.16*^{r}±36.83	100.00*^{r} ±0.00
GRP 4 25mg/kg (cadmium) + 7mg/kg (Amlodipine)	268.33*±15.83	263.72*±28.28	266.27*±30.36	153.55*^{r}±26.27	138.50*^{r}±42.34

Values are presented in mean ± standard error of the mean (n = 5), and * means values are statistically significant (p≤0.05) when compared to the control (p ≤0.05), while r means significant when compared with week zero.

Table 2: Result of Barnes Maze following treatment with various doses of Amlodipine.

GROUPS	WEEK 0	WEEK 1	WEEK 2	WEEK 3	WEEK 4
GRP 1 Control	17.38±40.54	66.94±49.88	40.38±29.80	45.05±37.12	45.05±37.12
GRP 2 25mg/kg (cadmium) +3mg/kg (Amlodipine)	225.05*±30.52	98.55a±37.73	72.94a±13.64	50.94±49.05	50.94a±49.05
GRP 3 25mg/kg (cadmium) +5mg/kg (Amlodipine)	187.11*±38.07	**115.44a±4.33**	222.61±31.81	167.88±8.26	67.88a±8.26
GRP 4 25mg/kg (cadmium) + 7mg/kg (Amlodipine)	165.55*±53.31	130.44a±24.54	208.0a5±8.64	108.38a ^{a}±31.61	108.38 ^{a} ±31.61

Values are presented in mean ± standard error of the mean (n = 5), and * means values are statistically significant (p≤0.05) when compared to the control (p ≤0.05), while r means significant when compared with week zero.

Table 3: Result of Passive avoidance test following various doses of amlodipine.

Groups	Week 0	Week 2	Week 3	Week 4
GRP 1 (Control)	1.20 ± 0.40	1.80 ± 0.40	2.0 ± 0.20	1.20 ± 0.40
GRP 2 25mg/kg (cadmium) + 3mg/kg (Amlodipine)	2.00 ± 0.00	1.60 ± 0.24	1.80 ± 0.37	2.80 ± 0.31
GRP 3 25mg/kg (cadmium) + 5mg/kg (Amlodipine)	2.80 ± 0.31	1.60 ± 0.24	1.80 ± 0.37	2.80 ± 0.31
GRP 4 25mg/kg (cadmium) + 7mg/kg (Amlodipine)	2.40 ± 0.60	1.80 ± 0.48	2.80 ± 0.73	2.40 ± 0.60

The data are shown as the mean standard error of the mean (n = 5), and values marked with an asterisk have a statistically significant difference from the control group (p 0.05).

Table 4: Result of Biomedical Analysis during for Total antioxidant capacity study.

GROUPS	TAC week 0	TAC Week 4
GRP 1 Control	1.40±0.07	1.61±0.2
GRP 2 25mg/kg (cadmium) + 3mg/kg (Amlodipine)	1.20*±0 .05	1.54[a]±01
GRP 3 25mg/kg (cadmium) + 5mg/kg (Amlodipine)	0.74*± 0 .01	1.02[a]±0.08
GRP 4 25mg/kg (cadmium) +7mg/kg (Amlodipine)	0.42* ± 0.08	2.65[a]±0.1

Values are presented in mean ± sem, n= 5. * means values are statistically significant (p≤0.05) when compared to the control, **a** means values are significant when compared to week zero.

Table 5: Result of Acetylcholinesterase and Nitric oxide.

GROUPS	ACH Week 0	ACH Week 4	Nitric Oxide week 0	Nitric Oxide week 4
GRP 1 Control	15.30±0.15	15.72±0 .15	4.36±0 .09	4.61±0.09
GRP 2 25mg/kg (cadmium) + 3mg/kg (Amlodipine)	18.29*±0.05	12.45a± 0.05	1.70±0 .01	1.77±0.01
GRP 3 25mg/kg (cadmium) + 5mg/kg (Amlodipine)	19.40*±0.36	11.40a±0 .36	0 .97±0 .01	1.00±0.01
GRP 4 25mg/kg (cadmium) + 7mg/kg (Amlodipine)	21.52*±0.20	9.00a± 0.20	0 .85±0 .03	0.95±0.03

Values are presented in mean ± sem, n= 5. * means values are statistically significant ($p \leq 0.05$) when compared to the control, **a** means values are significant when compared to week zero.

4. DISCUSSION OF FINDINGS

According to table 4.1 (Navigationa Maze test), there was significant increase in time of task performance in week 0 when compared to control group (i.e. after induction with Cadmium) this confirm the fact that cadmium successfully cause alteration in cognitive function in rats. When compared to the week Zero, the result revealed significant decrease in time of task performance in group 3 and group 4, at week 2 when compared to the control group and week zero during the test period. There was further significant decrease in time of task performance in Week 3 and Week 4 transversely the test groups.

As shown in table 4.2, the result of Barnes maze test showed that there was significant increase in task performance following treatment with various doses of Amlodipine when compared to the control group and week 0. Table 4.3 revealed the result of passive avoidance following administration of test substance to the various groups, the result revealed significant increase in avoidance time in Week 3 and Week 4 across the task groups when compared to the control group and week 0 of the test period.

The results are clear indication of cognitive dysfunction. After treatment, there was significant improvement in time of task performance. The result is in agreement with According to[26], abnormalities in navigation are indicative of pathological alterations in the basal ganglia and perhaps other cortical parts of the brain portion that are involved in learning, memory, and psychomotor action.

The result of total antioxidant capacity as shown in table 4.4 revealed that cadmium significantly decreased TAC when compared to the control group. However, at week 8 after administration at Amlodipine, there was significant increase across the test group when compared to the Week 0. Table 4.5, showed the result of Acetylcholinesterase and Nitric Oxide following 4 weeks of Administration of the test substance. From the result Acetylcholinesterase was significantly increased by the action of cadmium when compared to the control as seen at week 0. However, the administration of Amlodipine causes a decrease in AchE after 4 weeks of Administration. This result is consistent with the work of[27] who reported that Lead and cadmium in their singular form or in combination caused significant

accumulation of Acetylcholinesterase after a long period of exposure, they further reported that the distortion of the level of acetylcholinesterase in turn altered cognitive functions as well as motor activities in rats.

The result further revealed a significant decrease in Nitric Oxide after induction with Cadmium, However Amlodipine was able to reverse this effect after 8 weeks of Administration. Of course, it is established that, cadmium causes chromosomal deletions and mutations. Its harmful effects include reduced glutathione (GSH) depletion, protein binding of sulfhydryl groups, and increased generation of reactive oxygen species, including superoxide ion, hydrogen peroxide, and hydroxyl radicals.[28] On the other hand, amongst the possible other application of the drug, amlodipine (being a calcium channel blocker), is used to treat hypertension, or high blood pressure. It can help in preventing or reduce the incidences of severe forms of heart diseases and strokes; it is also used to stop angina, a kind of chest discomfort brought on by a heart condition.[29] Thus, in this study, this frequently prescribed and used antihypertensive, amlodipine, was used to see its possible ameliorative effects in the cognito-motor impairment of cadmium chloride toxicity.

The present study recorded a dose-dependent decrease in the navigation maze task. Considering the fact that the navigation maze task is helpful in the evaluation of exploration, route planning, and navigation, which depend on the formation of cognitive maps from learning and memory[30], it implies that the treatment with amlodipine may not have a remarkable amelioration on this impairment by the cadmium toxicity.

Further, the present also noted that there were dose-dependent increases in the Barnes maze tasks between weeks two and four of the treatment with amlodipine. Notably, the Barnes maze is a popular test for assessing spatial learning and memory in rats and mice.[31] The foregoing result thus implies that treatment was perhaps able to improve the negative impact of the cadmium toxicity on the mental representation of the study animals' environment. Even though the possible mechanism of achieving this outcome is not understood, this can be said to be a good attribute of the drug off from its primary use.

On further probe of the present study, it was found that the treatment with amlodipine in the cadmium intoxicated rats indicated decreases in passive avoidance test, total antioxidant capacity and AchE level in the study animals. It is thus suggestive to say that, although, amlodipine may be a potent a calcium channel blocker that helps in reducing vascular smooth

muscle constriction and helpful in reducing the incidences of severe forms of heart diseases, it may perhaps need higher doses show ameliorative effects on the above cadmium impaired parameters. Of course, such attribute is recommended to be confirmed by further studies before application in the study animals. Antihypertensive medications may be linked to lowering cognitive impairment, according to a number of observations, clinical investigations, and systematic examinations, however the outcomes of these research vary widely[32], our study revealed that amlidopine was able to meliorate the cognitive impairment caused by cadmium toxicity. The study is also consistent with the work of[33] who reported that Patients diagnosed with hypertension whom followed amlodipine saw a considerable reduction in their likelihood of experiencing a cardiovascular event such as a stroke or heart attack. According to the findings of this comprehensive study, the hazard ratio for amlodipine was lower than one for both stroke (0.69-1.04) and MI (0.77-0.98), which indicated that amlodipine was responsible for improved prevention of stroke and MI, this off course could be seen in the present study as amlodipine administration was able to improve motor activities.

5. CONCLUSIONS

From the result of the study it was thus resolved that exposure to cadmium toxicity has proven to cause negative alterations in memory and psychomotor activities in rats. Also, cadmium have considerable impact on the activities of Acetylcholinesterase and total antioxidant capacity. However, Amlodipine was able to restore the effects of cadmium on the studied parameters after 3 weeks of treatment.

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