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RESEARCH ARTICLE

Effects of Heavy Metal Toxicity on Anxiety Disorder

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ABSTRACT

Background: Several heavy metals are found naturally in the earth crust and are exploited for various industrial and economic purposes. Among these heavy metals, a few have impact on the human body. Though some of these metals only have effect on human physiology in high doses, others such as cadmium, mercury, lead, chromium, silver, and arsenic have delirious effects in the body even in minute quantities, causing acute and chronic toxicities in human. Anxiety is a common psychiatric disorder among men and women. Occasional anxiety may be a normal a part of life. However, people with anxiety disorders frequently have intense, excessive and persistent worry and fear about everyday situations. Often, anxiety disorders involve repeated episodes of sudden feelings of intense anxiety and fear or terror that reach a peak within minutes (panic attacks). This study examined a major environmental risk factor of anxiety disorder and how the adverse impact of anxiety disorder could be ameliorated using zinc and vitamin E.

Methods: Animals used for the researched were grouped into four. Group 1 represents control group; Group 2 represents animals exposed a heavy metal ($CdCl_2$); Group 3 represents animals exposed to $CdCl_2$ and then treated with zinc; and Group 4 represents animals exposed to CdCl2 and then treated vit E.

Results: Results showed that exposure to heavy metals ($CdCl_2$ in particular) causes severe anxiety disorders. Results also showed zinc and vit E have the capacity to ameliorate anxiety disorder caused by heavy metals.

Conclusion: The human body should be adequately protected as man interacts with heavy metals to prevent anxiety disorder, and where a patient becomes a victim of the disorder, zinc and vit E could be used to manage the case.

INTRODUCTION

Heavy metals are defined as metallic elements that have a comparatively high density compared to water. These heavy metals pollute the environment intensely and are in beginning areas like mining, foundries and smelters and other metal-based industrial operations [1,2].

Natural phenomena like weathering and volcanic eruptions have also been reported to significantly contribute to heavy metal pollution [3,4]. It has been said that metals such as Iron (Fe), Magnesium (Mg), Copper (Cu), to mention a few, are essential nutrients required for various biochemical and physiological functions. However, metals such as cadmium (Cd), Antimony (Sb), Lead (Pb), Vanadium (V) etc., have no established biological functions and are considered as non-essential metals yet they still find their way into the body system. Heavy metals have been reported to affect cellular organelles and components in biological systems [5].

Based on epidemiological and experimental studies showing an association

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between exposure and cancer incidence in humans and animals, cadmium has been named as either "known" or "probable" human carcinogen by the International Agency for Research on Cancer (IARC).

Cadmium chloride is a cadmium halide in the form of colourless crystals, soluble in water, methanol and ethanol. It is a ubiquitous environmental pollutant owing to its widespread use in industry throughout the world. It is a non- essential heavy metal with teratogenic, carcinogenic and mutagenic effects as a pollutant [6].

Cadmium is an environmental pollutant present in soil, water, air and food. Anthropogenic sources add 3-10 times more cadmium to the atmosphere than natural sources. It has a long biological half-life (between 15-30 years in humans) mainly due to its low rate of excretion from the body [7].

Contamination with this heavy metal occurs mainly through food intake, but tobacco smoke is also one of the major sources of exposure. It is released into water as a by-product of smelting, into air by combustion of coal and oil, and into soils as impurities [8].

Cadmium has been listed within the International Register Toxic Chemicals (IRPTC) of the United Nations Environment Program (IRPTC Legal file 1986, vol. 1) and the World Health Organisation (WHO) estimated 500 micrograms per week cadmium as the safe level for human ingestion [9].

Waste disposal contributes to largest input of cadmium to land compared to the other sources which include coal combustion, phosphate fertilizer manufacture and use, etc. [10]. Major sources of dietary cadmium are fish, liver, grains, leafy vegetables, potatoes and other root vegetables.

Cadmium chloride has various lines of applications and is mostly used industrially. The major industrial applications of cadmium include the production of alloys, pigments and batteries [11].

Invariably, people are exposed to cadmium on a daily basis. Cadmium is a toxic heavy metal with common exposure in industrial work places, plants, soils and from smoking. Due to its low permissible exposure to humans, over exposure may occur even in situations where trace quantities of cadmium are found.

However, the exposure to cadmium has its own dangers and effects on the creature being exposed to it. Cadmium has been reported to cause severe toxicity in various organs; mainly the kidney, liver, bone, lungs and genitals which leads to urinary, digestive, skeletal and reproductive system dysfunction and human health hazards [12].

Consequently, the International Agency for Research on Cancer (IARC) has classified cadmium as a group one human carcinogen. More severe exposures can affect the respiratory system causing shortness of breath, pneumonitis and pulmonary edema [13,14]. At peripheral level, prolonged exposure to cadmium may cause toxic effect due to its accumulation over time in a variety of tissues, including kidneys, liver, Central Nervous System (CNS) and peripheral neuronal systems. At the CNS, it could be transported through the blood-brain barrier and also from the nasal mucosa or olfactory pathways into the CNS. It acts as catalysts for biochemical reactions, regulators of gene expression, second messengers in signaling pathways and co-factors for vital enzymes notorious for regulating physiological, pathological and behavioural functions [15,16].

From studies, exposure to Cd is implicated in hyperactivity, increased aggression, impaired social memory processes and altered drinking behavior [17,18].

The hippocampus accumulates the divalent metals to a greater extent than do other parts of the brain. Behavioural alterations following heavy metal exposure have been related to hippocampal dysfunction. In this direction, animal studies involving Cd exposure also exhibit behavioural alterations. Rats exposed to Cd had decreased memory, as well as altered anxiety and fear responses [19].

Zinc

Zinc (Zn), required by many enzymes for their activities, is a trace element essential for living cells as it plays an important role in the DNA replication, transcription and protein synthesis influencing cell division and differentiation [20]. It functions in binding of specific genes with tetrahedral bonds that result in transcription, thereby directly involved in the translation step of gene expression of DNA elements.

Zinc deficiency may interfere with protein synthesis, leading to decrease in protein and accumulation of amino acids. This is because zinc is a structural component of the ribosomes and responsible for their structural integrity. Ribosomes disintegrate in the absence of Zn [20].

Zinc prevents cell damage through activation of the antioxidant system. It is an essential component of the antioxidant defense system and functions at many levels [21,22].

Vitamin E

Vitamin E is in the group of eight fat soluble chemicals. It is found in various foods and oils. Nuts seeds, vegetable oils, fortified cereal, and green vegetables are a major source of alpha-tocopherol and significant amounts are also available in green leafy vegetables and fortified cereals. Vit E from food sources is not known to be toxic. However, there are evidence of pro-oxidant damage associated with very high dose of Vit E supplements [23,24].

Vit E functions to prevent oxidative stress, protect cell

membranes, regulate platelet aggregation and protein kinase C activation and its role in disease prevention such as cancer, Alzheimer's disease, HIV/AIDS. Other theories hold that vit E act by controlling gene expression and cell signal transduction [25-27].

Anxiety

Anxiety is a general term for several disorders that cause nervousness, fear, apprehension and worrying. It is defined as an emotion characterized by feelings of tension, worried thoughts and physical changes like increased blood pressure. Anxiety is not the same as fear, which is a response to a real or perceived immediate threat; it is the expectation of future threat [28,29].

It is often accompanied by muscular tension, restlessness, fatigue and problems in concentration. Anxiety is generally not unusual, but when experienced regularly, the individual may suffer from an anxiety disorder. Anxiety disorders may be partly genetic but may also be due drug use, which are often prescribed to treat anxiety, as well as withdrawal from drugs of abuse [28].

Treatment options may involve lifestyle changes, medication, and therapy. Metacognitive therapy seeks to rid anxiety through reducing worry, which is seen as a consequence of metacognitive beliefs [30].

Seeing that biological organs and systems exposure to cadmium chloride may alter the functions of the systems, this study finds out how it affects the CNS to cause anxiety, and also the effect of zinc and vitamin E in ameliorating the effects on the anxiety levels.

MATERIALS AND METHODS

Animal's preparation

Twenty healthy CD1 mice, aged 8-10 weeks and weighing 18-30 g were used for the study. The animals were raised in the Faculty of Basic Medical Sciences Animal House, University of Calabar, Calabar, Nigeria. The animals were provided with food and water *ad libitum*. The food and water trough were changed daily while the beddings were changed after 3-5 days throughout the period of the experiment. They were kept in a room under standard conditions (temperature of 18-23°C and 40-60% humidity) and exposed to 12/12 hours light/dark cycle.

Experimental design

The 20 mice were randomly assigned into four (4) groups of five (5) animals;

Group A: Control

Group B: $CdCl_2$ only

Group C: $CdCl_2$ + Zinc

Group D: $CdCl_2$ + Vitamin E

Drug administration

The test drugs were reconstituted into appropriate concentrations as follows: 500 mg of $CdCl_2$ was dissolved in 10,000 ml of distilled water; (50 ppm). 400 mg of vitamin E was dissolved in 5ml of castor oil. 100 mg of zinc was dissolved in 69 ml of normal saline.

The drugs were administered orally with the aid of an orogastric cannula for a period of 14days. At the end of the treatment period, anxiety-like behaviours were evaluated following the Light and Dark Transition Box (LDTB) and Elevated Plus Maze (EPM) paradigms.

Determination of anxiety

The test for anxiety was done using:

1. Elevated Plus Maze (EPM)

2. Light and Dark Transition Box (LDTB)

Elevated Plus Maze (EPM): Elevated plus maze (EPM) is a test measuring anxiety in laboratory animals that uses rodents as a screening test for putative anxiolytic or anxiogenic compounds and as a general research tool in neurobiological anxiety research. In the EPM, this anxiety is expressed by the animal spending more time in the enclosed arms [31,32].

The test uses elevated, plus-shaped (+) apparatus with two open and two enclosed arms. The behavioural model is based on the general aversion of rodents to open spaces. This aversion leads to the behaviour termed thigmotaxis, a preference for remaining in enclosed spaces or close to the edges of a bounded space. In the EPM, this translates into the animals limiting their movement to the enclosed arms. Anxiety reduction is indicated in the plus maze by an increase in the proportion of time spent in the open arms (time in open arms/ total time in open or closed arms) and an increase in the proportion of entries into the open arms (entries into open arms/ total entries into open or closed arms). The total number of open arm entries and number of closed arm entries are sometimes used as measures of general activity [32,33].

The EPM was built according to the description of Lister (1987). The maze has two open arms (45 × 5cm²) with 0.25cm high edges and two closed arms (40 × 5cm²) with 15cm high walls radiating from a central square (5×5cm). The open arms contain a slight ledge (4 mm high) to prevent the mice from slipping and falling off the edge. The closed arms provide a sense of safety because they are enclosed like most tests of anxiety. This task exploits the conflict between the natural tendency of mice to explore the Novel areas and fear of open spaces. The index of open arm avoidance also gives a measure of anxiety [34].

Prior to the test, the plus maze arms surfaces and closed sides were cleaned with methylated spirit to eliminate olfactory cues and to remove fecal boli and urine. A mouse was placed in the central square of the plus maze such that the mouse faced an open arm away from the experimenter upon placement. Immediately after placement, a quiet stopwatch was started and mouse was allowed to explore the apparatus for five minutes. The test session was recorded. Behaviours such as open arm activity and head dipping were considered exploratory and a greater frequency of these measures shows a greater level of exploration [35].

Behaviors scored include;

- **Distance travelled:** total distance travelled by the animal during the trial.
- **Open arm entries:** frequency with which the animal entered the open arms. All four of the mouse's paws were required to be in the arm to be counted as an entry.
- **Closed arm entries:** frequency with which the animal entered the closed arms. All four of the mouse's paws were required to be in the arm to be counted as an entry.
- **Open arm duration:** duration of time the animal spent in the open arms.
- **Closed arm duration:** duration of time the animal spent in the closed arms.
- **Centre square entries:** frequency with which the animal entered the central square with all four paws.
- **Central square duration:** duration of time the animal spent in the central square.
- **Head dipping:** frequency with which the animal lowered the head over the sides of the open arm towards the floor.
- **Stretch - attend postures:** frequency with which the animal demonstrates forward elongation of head and shoulders followed by retraction to original position.
- **Rearing:** frequency with which the animal stands on hind legs or leans against walls of the maze with front paws.
- **Grooming:** duration of time the animal spent licking or scratching itself while stationary.
- **Urination:** number of puddles or streaks of urine.
- **Defecation:** number of fecal boli produced.

Light/Dark Transition Box (LDTB): The LDTB apparatus has two compartments. The light compartment is the 2/3 of the box and is brightly lit and open. The dark compartment is 1/3 of the total box and is covered and dark. A door of 7cm connects the two compartments. Rodents prefer dark areas over light areas. However, when presented in a novel environment, rodents have a tendency to explore. These two conflicting emotions lead to observable anxiety-like symptoms. Rodents typically spend more time in the dark compartment than the light compartment. If rodents are injected with anxiolytic drugs, percentage of time spent in the light compartment will increase. Locomotion and rearing, which is when the rodent stands up on its hind legs and is a sign of exploration in the dark compartment also increase. When injected with anxiogenic drugs, more time is spent in the dark compartment. The LDTB does not require any prior training. No food or water is deprived and only natural stressors such as light are used [36,37].

The light/dark box (45 × 27 ×27cm) is made of plywood and consists of two compartments of unequal sizes as described by Costall, et al. [3]. The smaller compartment (18 × 27cm) is painted black (2/5 of the box) and the larger compartment (27 × 27cm) is painted white (3/5 of the box). These compartments are connected by a door (7.5 × 7.5cm) located at the floor level in the centre of the wall between the two compartments. The floor is divided into 9 × 9 cm squares and is covered with Plexiglas. Both compartments are covered with lids of clear Plexiglas.

The mouse is placed in the light compartment of the apparatus and is allowed to move around. Typically the mouse will move around the periphery of the compartment until they find the door. The mouse is allowed to explore the apparatus for 5 minutes and the behaviours of the mouse in the box were recorded. All four paws must be placed into the opposite chamber to be considered an entry. After this, the mouse is removed and the box is cleaned using a cotton wool and 70% ethyl alcohol and allowed to dry between tests.

Behaviors scored [38,39];

- **Transitions:** number of times the animal passes into the opposite compartment. (All four paws of the mouse must move into the new compartment for a transition to be scored and for that compartment to be considered entered).
- **Line crosses:** number of times the animal crossed a line drawn on the floor of the box.
- **Rearing:** frequency with which the animal stands on its hind legs or leans against the wall of the box with front paws.
- **Stretch - attend postures:** frequency with which the animal demonstrates forward elongation of head and shoulders followed by retraction to original position.
- **Grooming:** duration of time the animal spent licking the body while stationary.
- **Dark box duration:** length of time the animal spent in the dark side of the box.

- **Light box duration:** length of time the animal spent in the light side of the box.
- **Defecation:** number of fecal boli produced (light vs dark).
- **Urination:** number of puddles or streaks of urine (light vs dark).

Statistical analysis

Data obtained from the tests were analysed and results were presented in the form of graphs of means ± Standard Error of Mean (SEM). Analysis of Variance (ANOVA) and a post- hoc student t- test were used to test for significant variability between the test and control groups. The probability level $p < 0.05$ was accepted as significant.

RESULTS

Comparison of frequency of rearing among the different experimental groups in the light/dark transition box

The mean ± SEM rearing frequency for the control, $CdCl_2$ only, $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were 59.60 ± 5.12, 35.40 ± 3.41, 55.40 ± 3.04 and 53.60 ± 3.66 respectively.

The results showed that the rearing frequency in the $CdCl_2$ was significantly lower ($p < 0.05$) when compared with the control group. However, the rearing frequency of the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were significantly higher ($p < 0.05$) when compared with the $CdCl_2$ group. .

Comparison of frequency of stretch attend posture (SAP_{LDT}) among the different experimental groups in the light/dark transition box

The mean ± SEM SAP frequency for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were 2.00 ± 0.71, 3.60 ± 0.51, 1.60 ± 0.51 and 1.60 ± 0.51 respectively.

The results showed that the SAP frequency in the $CdCl_2$ group had no significant difference when compared with the control group. However, the SAP frequency in the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were significantly lower ($p < 0.05$) when compared with the $CdCl_2$ group.

Comparison of frequency of Transition between the Light/Dark (TLD) compartments among the different experimental groups in the light/dark transition box (Table 1)

The mean ± SEM frequency of transition for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were 21.60 ± 2.01, 7.40 ± 1.03, 17.40 ± 1.63 and 21.40 ± 2.01 respectively.

The results showed that the frequency of transition in the $CdCl_2$ group was significantly lower ($p < 0.05$) compared with the control group. However, the frequency of transition in the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were significantly higher ($p < 0.05$) when compared with the $CdCl_2$ group.

Comparison of Dark Duration in Light/Dark (DDLD) transition box test among the different experimental groups

The mean ± SEM dark duration for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were 85.00 ± 12.98, 213.50 ± 17.06, 81.63 ± 15.87 and 63.76 ± 18.35 respectively.

The results showed that the duration in the dark chamber for the $CdCl_2$ group was significantly higher ($p < 0.05$) when compared with the control group. However, the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups had a significantly lower ($p < 0.05$) dark chamber duration when compared with the $CdCl_2$ group.

Comparison of Light Duration during Light/Dark (LDLD) transition box test among the different experimental groups

The mean ± SEM light duration for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were 215.00 ± 12.98, 86.55 ± 17.09, 218.37 ± 15.87 and 236.24 ± 18.35 respectively.

The results showed that the duration in the light chamber for the $CdCl_2$ group was significantly lower ($p < 0.05$) when compared with the control group. However, the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups had significantly higher ($p < 0.05$) light chamber duration when compared with the $CdCl_2$ group.

Comparison of the frequency of grooming during light/dark transition box test among the different experimental groups (Table 2)

The mean ± SEM grooming frequency for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were 1.80 ± 0.37, 7.20 ± 0.86, 2.60 ± 1.08 and 4.20 ± 0.58 respectively.

Table 1: Showing comparison of frequency of Rearing, frequency of Stretch Attend Posture (SAP), frequency of Transition between the Light/Dark (TLD), Dark Duration in Light/Dark (DDLD) transition box test, Light Duration during in Light/Dark (LDLD) transition box test among different groups.

Groups	Rearing	SAP	TLD	DDLD	LDLD
Control	59.60 ± 5.12	2.00 ± 0.71	21.60 ± 2.01	85.00 ± 12.98	215.00 ± 12.98
$CdCl_2$ only	35.40 ± 3.41	3.60 ± 0.51	7.40 ± 1.03	213.50 ± 17.06	86.55 ± 17.09
$CdCl_2$ + Zinc	55.40 ± 3.04	1.60 ± 0.51	17.40 ± 1.63	81.63 ± 15.87	218.37 ± 15.87
$CdCl_2$ + vit E	53.60 ± 3.66	1.60 ± 0.51	21.40 ± 2.01	63.76 ± 18.35	236.24 ± 18.35

Table 2: Showing comparison of the frequency of Grooming during Light/Dark Transition Box Test, frequency of Rearing during Elevated Plus Maze (REPM), frequency of Stretch Attend Posture in the Elevated Plus Maze Test (SAP_{EPM}), frequency of Head Dips in the Elevated Plus Maze Test (HD_{EPM}), and frequency of Grooming during Elevated Plus Maze among different experimental groups.

Groups	Grooming (LDT)	REPM	SAP_{EPM}	HD_{EPM}	Grooming Freq. (EPM)
Control	1.80 ± 0.37	40.60 ± 3.89	7.60 ± 1.54	11.00 ± 2.70	2.60 ± 0.40
$CdCl_2$ only	7.20 ± 0.86	23.20 ± 3.73	11.00 ± 1.26	14.40 ± 1.72	7.20 ± 0.58
$CdCl_2$ + Zinc	2.60 ± 1.08	35.00 ± 3.54	7.00 ±1.64	8.00 ± 1.22	2.20 ± 0.58
$CdCl_2$ + vit E	4.20 ± 0.58	40.40 ± 1.86	4.00 ± 0.71	9.80 ± 1.16	1.80 ± 0.37

The results showed that the grooming frequency in the $CdCl_2$ group was significantly higher ($p < 0.05$) when compared with the control group. However, the grooming frequency in the $CdCl_2$ + Zinc group was significantly lower ($p < 0.05$) when compared with the $CdCl_2$ group.

Comparison of the frequency of Rearing during Elevated Plus Maze (REPM) test among the different experimental groups

The mean ± SEM rearing frequency for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + Vit E groups were 40.60 ± 3.89, 23.20 ± 3.73, 35.00 ± 3.54 and 40.40 ± 1.86 respectively.

The results showed that the rearing frequency in the $CdCl_2$ group was significantly lower ($p < 0.05$) when compared with the control group. However, the rearing frequency in the $CdCl_2$ + Vit E group was significantly higher ($p < 0.05$) when compared with the $CdCl_2$ group.

Comparison of the frequency of Stretch Attend Posture (SAP_{EPM}) in the elevated plus maze test among the different experimental groups

The mean ± SEM SAP frequency for the control, $CdCl_2$, $CdCl_2$ + Zinc, $CdCl_2$ + Vit E groups were 7.60 ± 1.54, 11.00 ± 1.26, 7.00 ± 1.64 and 4.00 ± 0.71 respectively.

The results showed that the SAP frequency in the $CdCl_2$ group had no significant difference when compared with the control group. However, the SAP frequency in the $CdCl_2$ + Vit E was significantly lower ($p < 0.05$) when compared with the $CdCl_2$ group.

Comparison of the frequency of head dips in the elevated plus maze test among the different experimental groups

The mean ± SEM head dips frequency for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E were 11.00 2.70, 14.40 ± 1.72, 8.00 ± 1.22 and 9.80 ± 1.16 respectively.

The results showed that the frequency of head dips did not significantly differ between groups.

Comparison of the frequency of grooming during elevated plus maze test among the different experimental groups

The mean ± SEM grooming frequency for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + Vit E groups were 2.60 ± 0.40, 7.20 ± 0.58, 2.20 ± 0.58 and 1.80 ± 0.37 respectively.

The results showed that the grooming frequency in the $CdCl_2$ group was significantly higher ($p < 0.05$) when compared with the control group. However, the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups had significantly lower ($p < 0.05$) grooming frequency when compared with the $CdCl_2$ group.

Comparison of grooming duration in the elevated plus maze test among the different experimental groups

The mean ± SEM grooming duration for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + Vit E groups were 10.71 ± 2.79, 38.08 ± 5.61, 11.49 ± 3.33 and 7.39 ± 1.99 respectively.

The results showed that the duration of grooming in the $CdCl_2$ group was significantly higher ($p < 0.05$) when compared with the control group. However, the $CdCl_2$ + Zinc and $CdCl_2$ + Vit E groups had significantly lower ($p < 0.05$) grooming duration when compared with the $CdCl_2$ group. .

Comparison of the frequency of closed arm entry during elevated plus maze test among the different experimental groups

The mean ± SEM frequency of closed arm entry for

Table 3: Showing comparison of the Grooming duration in the Elevated Plus Maze Test, frequency of Closed Arm Entry during Elevated Plus Maze Test, Duration in the Closed Arm Entry During (CAE Dur.) Elevated Plus Maze Test, frequency of Open Arm Entry (Freq. OAE) during Elevated Plus Maze test, Duration in the Open Arm During Elevated Plus Maze Test among different experimental groups.

Groups	$Grooming_{(EPM)}$ Dur.	Freq. CAE_{EPM}	CAE_{EPM} Dur.	Freq. OAE_{EPM}	OAE_{EPM} Dur.
Control	10.71 ± 2.79	3.80 ± 0.80	112.27 ± 10.92	9.40 ± 0.75	185.73 ± 10.66
$CdCl_2$ only	38.08 ± 5.61	5.80 ± 0.66	238.29 ± 18.21	2.80 ± 0.37	61.71 ± 18.21
$CdCl_2$ + Zinc	11.49 ± 3.33	3.00 ±0.45	144.29 ± 7.96	8.20 ± 0.86	155.71 ± 7.96
$CdCl_2$ + vit E	7.39 ± 1.99	3.20 ± 0.58	106.34 ± 26.04	8.60 ± 0.51	193.66 ± 26.04

control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were 3.80 ± 0.80, 5.80 ± 0.66, 3.00 ±0.45 and 3.20 ± 0.58 respectively.

The results showed that the frequency of closed arm entry in $CdCl_2$ group had no significant difference when compared with the control group. However, the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups had significantly lower ($p < 0.05$) frequency of closed arm entry when compared with the $CdCl_2$ group.

Comparison of the duration in the Closed Arm Entry During (CAE Dur.) elevated plus maze test among the different experimental groups

The mean ± SEM closed arm duration for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E were 112.27 ± 10.92, 238.29 ± 18.21, 144.29 ± 7.96 and 106.34 ± 26.04 respectively.

The results showed that the duration in the closed arm in $CdCl_2$ group was significantly higher ($p < 0.05$) when compared with the control group. However, the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups had significantly lower ($p < 0.05$) duration in the closed arm when compared with the $CdCl_2$ group. .

Comparison of the frequency of Open Arm Entry (Freq. OAE) during elevated plus maze test among the different experimental groups (Table 3)

The mean ± SEM frequency of open arm entry for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups were 9.40 ± 0.75, 2.80 ± 0.37, 8.20 ± 0.86 and 8.60 ± 0.51 respectively.

The results showed that the open arm entry frequency in the $CdCl_2$ group was significantly lower ($p < 0.05$) when compared with the control group. However, the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups had significantly higher ($p < 0.05$) open arm entry frequency when compared with the $CdCl_2$ group.

Comparison of the duration in the open arm during elevated plus maze test among the different experimental groups

The mean ± SEM open arm duration for the control, $CdCl_2$, $CdCl_2$ + Zinc and $CdCl_2$ + vit E were 185.73 ± 10.66, 61.71 ± 18.21, 155.71 ± 7.96 and 193.66 ± 26.04 respectively.

The results showed that the duration in the open arm in the $CdCl_2$ group was significantly lower ($p < 0.05$) when compared with the control group. However, the $CdCl_2$ + Zinc and $CdCl_2$ + vit E groups had significantly higher ($p < 0.05$) open arm duration when compared with the $CdCl_2$ group.

DISCUSSION

Anxiety is a general term used to describe severe disorders that cause nervousness, fear, apprehension and worrying [28]. The Light and Dark Transition Box (LDTB) and the Elevated Plus Maze (EPM) tests were used to assess anxiety. The frequencies and duration of grooming, rearing and stretch attend posture were all markers used in the assessment of anxiety.

Increased duration in the light box compartment shows less anxiety and increased duration in the dark box compartment shows increased anxiety [37].

The results of this experiment in the light/dark transition box test showed that the control group had decreased dark chamber duration and an increased duration in the light chamber in the control group. This indicates that the anxiety level of the mice that were not exposed to $CdCl_2$ was low.

But there was an increased duration in the dark chamber and decreased light chamber duration in the group exposed to $CdCl_2$, indicating that exposure to $CdCl_2$ increased anxiety.

Also, the zinc and vitamin E treated groups that were earlier exposed to $CdCl_2$, had a decreased duration in the dark chamber and an increased duration in the light chamber. This implies that zinc and vit E have the capacity to decrease anxiety even after exposure to conditions that had previously increased it.

Increase in frequency of grooming shows increased levels of anxiety [40].

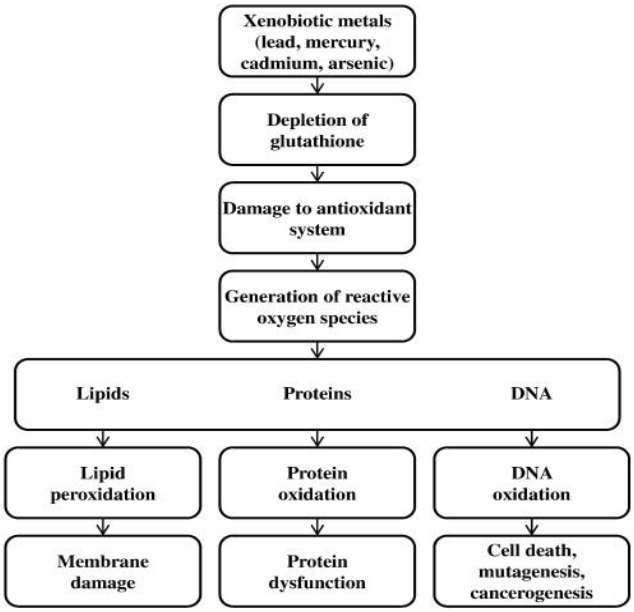
The results of this experiment showed a higher grooming frequency in the $CdCl_2$ exposed group when compared with control group confirming increased anxiety with exposure to $CdCl_2$ and a lower grooming frequency in the zinc and vit E treated group. This also confirms that zinc and vit E reduces anxiety.

Anxiety is expressed by the animal spending more time in the enclosed arm; denoting that increased duration in the closed arm indicates increased anxiety level and an increased duration in the open arm indicates decreased anxiety levels [32].

The results of this experiment in the elevated plus maze test showed that the $CdCl_2$ group had a higher duration in the closed arm of the maze and a lower duration in the open arm when compared with the control group; while the zinc and vitamin E treated group had a lower duration in the closed arm and a higher duration in the open arm of the maze. It also showed a higher grooming frequency and duration in the $CdCl_2$ group when compared with the control group; and a lower grooming frequency and duration in the zinc and vitamin E treated groups. This further confirms that exposure to Cadmium Chloride ($CdCl_2$) causes anxiety in mice. It also indicates that Zinc and vitamin E which are major antioxidants help to reduce anxiety (stress) [21,25].

The main mechanism of heavy metal toxicity include the generation of free radicals to cause oxidative stress, damage of biological molecules such as enzymes, proteins,

lipids, and nucleic acids, damage of DNA which is key to carcinogenesis as well as neurotoxicity.



CONCLUSION

The results of this study suggest that exposure to cadmium chloride ($CdCl_2$) causes anxiety in CD1 mice, and zinc and vitamin E through their antioxidant property lower anxiety levels in CD1 mice after its exposure to Cadmium chloride ($CdCl_2$).

ETHICAL CLEARANCE

The Name of the Ethic Committee that approved our work is: "University of Calabar Faculty of Medical Sciences Ethics Committee on Animals Experiments". The reference/approval number for the research work is: UCFMSECAE1047.

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