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Comparative anxiolytic, motor coordination and antioxidant effects of vitamin C and rutin on Cadmium-induced neurotoxicity in Swiss white mice

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Abstract

Cadmium a common environmental pollutant and induces neurotoxicity by mechanisms including oxidative stress. This study therefore evaluated the possible effects of common antioxidants, vitamin C and Rutin on Cadmium-induced neurotoxicity in mice. Twenty-five Albino mice weighing 18-28g used for the study were randomly assigned into 5 groups namely, control, Cadmium-only, Cadmium+vitamin C, Cadmium+Rutin and Cadmium+Vitamin C+Rutin groups. After 21 days of administration, neurobehavioural evaluation of the mice was carried out, animals sacrificed and the brain excised for biochemical analysis. Results show a significant increase in foot slips in the Cadmium-only group compared with control ($p<0.05$) but differentially lower in all treated than in the Cadmium-only groups ($p<0.05$). Stretch attend posture was significantly reduced in the Cadmium+VitaminC+Rutin group compared with the control and Cadmium-only groups ($p<0.05$). Open arm entry duration was significantly decreased in the Cadmium+Vitamin C compared with control ($p<0.05$) groups. Duration of closed arm entry was significantly higher in the Cadmium+Vitamin C compared with the Cadmium-only groups. Dark box duration in the Cadmium+Rutin group was significantly reduced compared with Cadmium+vitamin C groups ($p<0.05$) and significantly higher in the Cadmium+Rutin than in the Cadmium+vitamin C groups in the light box duration. Brain concentrations of malondialdehyde were significantly increased in the Cadmium only compared with control ($p<0.05$) compared with Cadmuim-only group and lower in Cd+vitamin C than Cd+Rutin. Catalase, superoxide dismutase and glutathione peroxidase activities activities were significantly decreased ($p<0.05$) in Cadmium-only group compared with control but increased in Cadmium+vitamin C and Cadmium+vitamin C+Rutin compared with Cadmium-only groups, and higher in Cd+Vitamin C than Cd+Rutin. We therefore conclude that Vitamin C may may play a more significant role in mitigating Cd-induced incoordination and oxidative stress in the mice than Rutin.

Keywords: Vitamin C; Rutin; anxiety; Motor coordination; Cadmium; Neurotoxicity; Comparative

1. Introduction

Cadmium (Cd) is a naturally occurring metal usually found as impurity in Zinc or Lead deposits. Cadmium is widely dispersed in different applications. It is used in the manufacturing of plastics, cigarette, petrochemicals, ceramics, paints and fireworks among others [1]. It also finds its application as a coating material in PVC and ship building industry.

Exposure to Cadmium is by both industrial and non-industrial means and in the production of battery, from fossil fuel as well as leakage from sewage sludge. These sources make Cadmium an easy environmental pollutant. Humans get exposed to Cadmium through drinking water and food contaminated by these products or through inhalation of dust or air that have been contaminated by industrial activities, exhaust fumes or cigarette [2, 3].

Cadmium-induced cytotoxicity has been well documented. Exposure to Cadmium has been associated with hepatotoxicity, nephrotoxicity cardiovascular toxicity, osteotoxicity [4] and reproductive toxicity [5]. Cadmium also

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causes neurotoxicity associated with cognitive impairment, learning and memory defects [6,7,8] and loss of equilibrium [9].

Cadmium (Cd) induces cytotoxicities by several mechanisms including induction of apoptosis, alteration in gene expression and inhibition of cellular respiration [10]. Cadmium also causes cytotoxicity by induction of oxidative stress, an imbalance between generation of reactive oxygen species and the scavenging or antioxidant system of the body [9,11].

Vitamin C (Abscorbic acid) is a water-soluble antioxidant vitamin which plays an essential role in tissue repairs, formation of collagen and enzymatic synthesis of some neurotransmitters [12]. Though Vitamin C can be synthesized by animals, it is commonly found in citrus and other fruits, barriers and vegetables [13,14]. Vitamin C (Vit C) is a potent and readily available antioxidant that neutralized free radicals and so, protecting cells against oxidative stress. This has been demonstrated in several studies, both in vivo and in vitro [15,16,17,18].

Rutin is a plant-based flavonoid and pigments with several health-related benefits. It is a glycoside with structure similar to Quercetin [19,20]. Plants including buckwheat, citrus fruits, apples, tea, red wine and vegetable are rich sources of Rutin [21]. Rutin has anti-inflammatory and antioxidant activities [22].

To evaluate central nervous system functions, some neurbehavioral indices are used. Neurbehavior refers to any behavioral response that arises from processing of the central nervous system and it encompasses cognitive and perceptual aspects of behavior including attention, learning, memory, spatial relationship and emotion and coordination [23]. In lower animals, neurbehavioral assessments are carried out using apparatuses like the Morris water maze, for spatial learning, open field test for general activity and anxiety, the light/dark transition box and the elevated Plus maze for anxiety-like behaviors and the Beam walking apparatus for motor coordination [24,25]

2. Material and methods

2.1. Experimental animals

Twenty five Albino mice weighing about 18-28g were used for the study. The mice were housed in the Animal house of the Department of Physiology, University of Calabar under a 12 hour light/dark cycle. They were kept in plastic cages under standard care as stipulated in the standards relating to the care and management of laboratory animals. The mice were first acclimatized for one week and also allowed free access to water and rat feed throughout the 21days duration of experimentation.

2.2. Collection and preparation of drugs

Rutin was bought from Swanson Health Products, USA while normal saline (0.9%) and Vitamin C were obtained from Bez Pharmacy, Calabar. All drugs solutions were prepared by dissolving them in 0.9% normal saline.

2.3. Experimental design

The animals were randomly assigned into 5 groups of 5 rats each. The groups were, control, Cadmium-only, Cadmium+Vitamin C, Cadmim+Rutin and Cadmium+Vitamin C+Rutin. Cadmium was given intraperitoneally at a dose of 1.5mg/kg to induce neurotoxicity. Vitamin C was administered orally every day at a dose of 10mg/kg while Rutin was given intraperitoneally and daily at a dose of 5mg/kg. Duration of treatment was 21 days. Cadmium in all the groups was administered daily for 7 days before commencement of treatment with Rutin and Vitamin C and then administered every 2 days. After the 21 days of treatment, animals were evaluated neurobehaviorally as documented below,

2.4. Collection and biochemical analysis of brain tissue

At the end of the study period or evaluation, animals were sacrificed and their brains dissected out. The brain tissues were homogenized in 0.1M sodium phosphate buffer (pH 7.4). The homogenates were then centrifuged at 10,000rpm for 10 minutes and stored at 20^0C. The supernatants were then used for biochemical assays. Superoxide activity was assayed using the method described by Marklund [26] while catalase activity was determined using the method of Aebi [27]. Glutathione peroxidase activity was evaluated as described by Rice-Evans [28] while malondialdehyde concentration was assayed as described by Jiang et al., [29].

2.5. Behavioural evaluation

2.5.1. Anxiety/Fear

Anxiety and fear were determined using the light and dark transition box and the elevated plus maze tests.

Light and dark transition test

The light box transition test (LBT) is one of the most widely used-tests to measure anxiety-like behaviors in mice. The test apparatus consists of a box divided into small (one third) dark chamber and a large (two thirds) brightly illuminated chamber [30]. Mice are normally placed into the lit compartment and allowed to move freely between the two chambers [31]. The first latency to enter the dark compartment and the total time spent in it are indications of bright space anxiety. Transitions are indices of activity/exploration because of habituation over time.

The procedure was carried out as demonstrated by Costall et al [32]. Mice were picked at the bases of their tails and placed at the center of the white compartment facing the door and allowed to explore the apparatus for 5 minutes and then removed and the maze cleaned with 70% alcohol solution which is allowed to dry to eliminate olfactory clues. Behaviors scored included stretch attend posture in dark and light transition box, dark box duration and light box duration.

Elevated plus maze (EPM) test

This test is used for measuring anxiety levels. The model is based on the animal's aversion to open spaces and tendency to be thigmotaxic [33]. The maze is shaped like a 'Plus' or '+'. The maze was built as described by Lister [34] with the arm having a slight age to prevent the mice from falling off [35]. Mice were handled at the base of their tails and placed in the maze for 5 minutes while its behavior is observed. Behaviors observed included head dips, stretch attend posture, open arm entry duration and frequency, close arm entry frequency and duration.

2.5.2. Test for Coordination

Beam walking test

This test is used to assess motor coordination. The apparatus is a narrow wooden beam (100 cm long) coated with black paint and elevated to a height of 40cm between a pole and their home cage (to attract the mice to the finished point). The beam was marked with lines at 5cm intervals. A healthy animal is expected to traverse the beam without support. Each mice was moved from the home cage and placed at the other end of the balance beam and the mice allowed to move towards the home cage for 2 minutes trial. Parameters evaluated include, reversals (frequency of times animal reverses) and foot slips. Test was done using a dim light under a camera coverage placed 175cm above the beam.

2.6. Statistical analysis

Data obtained was expressed as mean$\pm$SEM and analyzed using one way analysis of variance (ANOVA) followed by a post hoc Neuma –Keuls test. A p-value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Light and dark transition box test

3.1.1. Comparison of stretch attend posture

The values for control, Cd–only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin were 2.0$\pm$0.95, 1.8$\pm$0.73, 1.4$\pm$0.67, 0.8$\pm$0.8 and 0.4$\pm$0.4 respectively. There were no significant differences among the groups as shown in table 1.

3.1.2. Comparison of dark box duration in the light dark transition box test

The values for mean duration were 171$\pm$6.28, 162.79$\pm$20.44, 197.16$\pm$18.99, 132.39$\pm$12.46 and 171.75$\pm$7.39 for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin groups respectively. It was significantly reduced ($p<0.05$) in the Cd+Rutin group compared with Cd+vit C group as shown in table 1.

3.1.3. Comparison of light box duration in the light dark transition box test

The values for the mean duration were 129±6.25, 137.20 ± 20.44, 102.84 ± 18.92, 167.60 ± 12.46 and 128.25 ±7.39 for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin groups respectively. It was significantly increased in the Cd+Rutin group compared with Cd+vit C group as shown in table 1.

Table 1 Light and dark transition box test in different experimental groups

Parameter	Control	Cd-only	Cd+Vit C	Cd+Rutin	Cd+VitC+Rutin
Stretch attend posture	2±0.95	1.8±0.73	1.4±0.67	0.8±0.8	0.4±0.4
Dark box duration	171±6.25	162.79±20.44	197.16±18.92	132.39±12.46^{c}	171.75±7.39
Light box duration	129±6.25	137.20±20.44	102.84±18.92	167.60±12.46^{c}	128.25±7.39

Light and Dark Transition Box. Values are mean±SEM, n=5 c = p<0.05 vs Cd+Vit C

3.2. Elevated plus maze test

3.2.1. Comparison of head dips in different experimental groups

The values for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin groups were 6.6±1.72, 8.0±6.0, 5.4±1.75, 13.2±3.38 and 15.2±4.13 respectively. There were no significant differences among the groups as shown in Table 2.

3.2.2. Comparison of stretch attend posture in EPM

The values for control, Cd-only, Cd+vit C, Cd+Rutin, and Cd+vitC+Rutin, were 7.8±1.46, 7.4±1.93, 6.8±1.72, 4±0.32 and 2.4±1.69 respectively. It was significantly reduced in the Cd±vit C+Rutin compared with control and Cd-only groups (P<0.05) as shown table 2

3.2.3. Comparison of open arm entry frequency

The values obtained for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin were 2.8±0.58, 2.2±0.48, 1.6±0.51, 2.6±0.4 and 4.2±0.97 respectively. It was significantly increased (P<0.05) in the Cd+vitC+Rutin group compared with control and Cd-only groups as shown in table 2.

3.2.4. Comparison of open arm entry duration in EPM

The values were 34.83±10.34, 78.54±29.27, 21.65±11.22, 465.46±11.82 and 76.8 ±24.00 for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin respectively. It was significantly reduced in the Cd+vit C compared with Cd–only groups as shown in table 2.

3.2.5. Comparison of close arm entry frequency

The result shows that, the values for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin were 3.2±0.49, 2.2±0.49, 1.6±0.45, 3.0±0.32 and 4.6±1.12 respectively. It was significantly increased in the Cd+vitC+Rutin group compared with Cd–only and Cd+vit C groups as shown table 2.

3.2.6. Comparison of close arm entry duration

The values for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin were 265.17±10.84, 221.45±29.27, 27.31±11.20, 253.53±11.82 and 223.42±24.12 respectively. It was significantly higher in the Cd+Rutin than in the Cd+vit C groups as shown in table 2.

Table 2 Elevated plus maze (EPM) tests in different experimental groups

Parameter	Control	Cd-only	Cd+Vit C	Cd+Rutin	Cd+VitC+Rutin
Head dips	6.6±1.72	8±6.00	5.4±1.75	13.2±3.38	15.2±4.13
Stretch attend posture	7.8±1.46	7.4±1.93	6.8±1.72	4±0.32	2.4±1.69[ab]
Open arm entry frequency	2.8±0.58	2.2±0.48	1.6±0.51	2.6±0.4	4.2±0.97/5[bc]
Open arm entry duration	34.83±10.84	78.54±29.27	21.65±11.22[b]	46.46±11.82	76.18±24.00
Close arm entry frequency	3.2±0.49	2.2±0.49	1.6±0.4	3±0.32	4.6±1.12/5[bc]
Close arm entry duration	34.83±10.84	78.54±29.27	21.65±11.22[b]	46.46±11.82	76.18±24.00

3.2.7. Comparison of open and close frequency and duration in different groups

Values are mean±SEM, n=5

- a = p<0.05 vs Control
- b = p<0.05 vs Cd - only
- c = p<0.05 vs Cd+Vit C

3.3. Beam walking

3.3.1. Frequency of reversal in different experimental groups during beam walking test.

The frequency of reversal for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin were 5.6±1.53, 3.2±0.58, 2.4±1.12, 3.6±1.12 and 3.8±0.92 respectively. It was significantly reduced in the Cd+Vit C compared with control (P<0.05) as shown in table 3.

3.3.2. Comparison of foot slips between different experimental groups

The values were 0.4±0.24, 3.22±0.37, 0.4±0.24, 0.8±0.37 and 0.2±0.2 for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+Rutin+vit C groups respectively. It was significantly increased in Cd-only group compared with the control but lower in all treated groups than in the Cd-only group as shown in table 3.

Table 3 Beam walking in different experimental groups

Parameter	Control	Cd-only	Cd+Vit C	Cd+Rutin	Cd+VitC+Rutin
Frequency of reversal	5.6±1.33	3.2±0.58	2.4±1.12[a]	3.6±1.12	3.8±0.92/5
Foot slips	0.4±0.24	3.2±0.37[a]	0.4±0.24[b]	0.8±0.37[b]	0.2±0.2[b]

Frequency of reversal and foot slip in experimental groups.

Values are mean±SEM, n=5

- a = p<0.05 vs Control
- b = p<0.05 vs Cd-only

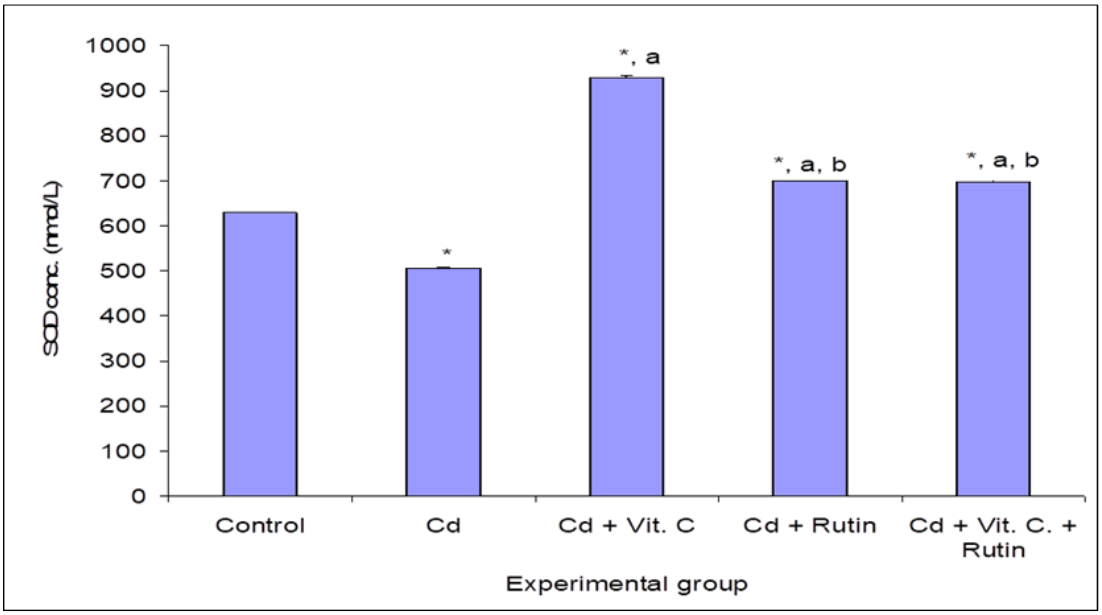
3.4. Comparison of oxidative stress biomarkers in different experimental groups

3.4.1. Superoxide dismutase (SOD) activity

The values for SOD activity were 628.7±0.54, 505.43±2.07, 929±4.65, 699.77±0.52 and 696.77±1.29 for control, Cd–only, Cd + vit C, Cd+Rutin and Cd+vitC+Rutin groups respectively. There was a significant decrease in SOD activity in Cd–only compare with control groups (P<0.05). The values were significantly increased in all treated groups compared with Cd-only and control groups (P<0.05). It was however significantly lower in the Cd+Rutin and Cd+vitC+Rutin groups than in the Cd+vit C group as shown in Fig 1.

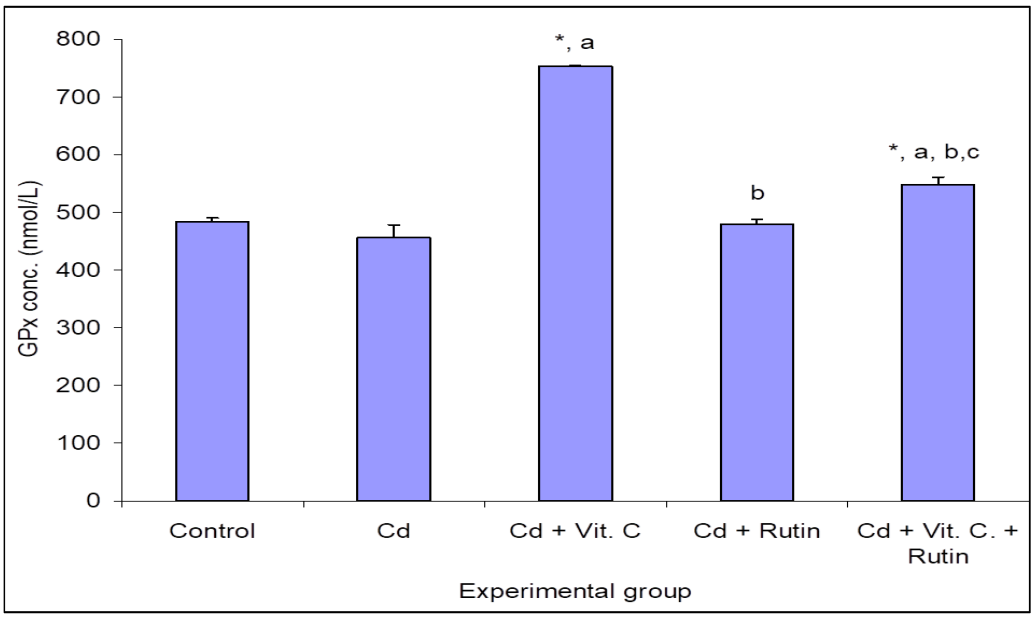
3.4.2. Glutathione peroxidase (GPx) activity

The activities of GPx in the different groups were 483.1±6.58, 456.33±21.66, 752.87±1.03, 479.547±7.57, and 548.47±12.37 for control, Cd–only, Cd+Vit C, Cd+Rutin and Cd+vitC+Rutin groups respectively. It was significantly increased in the Cd+vit C and Cd+vitC+Rutin compared with the control and Cd-only groups. It was also significantly higher in the Cd+vitC+Rutin than in the Cd+vit C groups as showed in Fig 2.



Values are expressed as mean + SEM,; n= 5; *=p<0.05 vs control; a= p<0.05 vs cd; b = p< 0.05 vs cd. Vit,c

Figure 1 Comparison of superoxide dismutase concentration in the different experimental groups



Values are expressed as mean + SEM, n= 5; *=p<0.05 vs control; a= p<0.05 vs cd +vit,c; b = p< 0.05 vs cd rutin

Figure 2 Comparison of glutathione peroxidase concentration in the different experimental groups

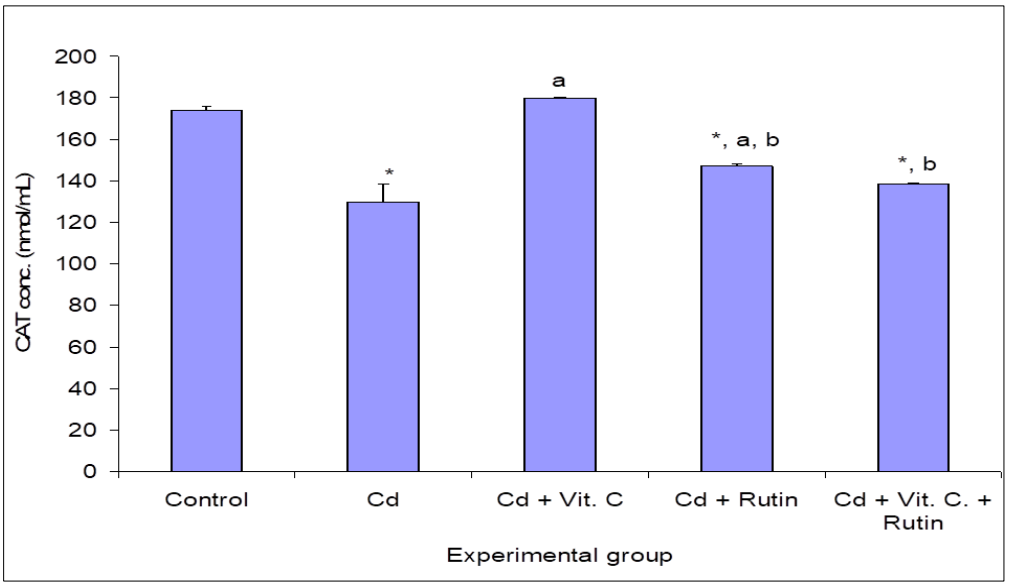
3.4.3. Catalase (CAT) activity

Catalase activities were 174.17±1.58, 129.97±0.54, 179.9±0.31, 147.1±1.16 and 138.43±0.65 for control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin groups respectively. It was significantly decreased in the Cd-only, Cd+Rutin and

Cd+vit+Cd+Rutin groups compared with control (p<0.05), though significantly higher in the Cd+vit C and Cd+Rutin groups than in the Cd-only group. This is shown in Fig 3.

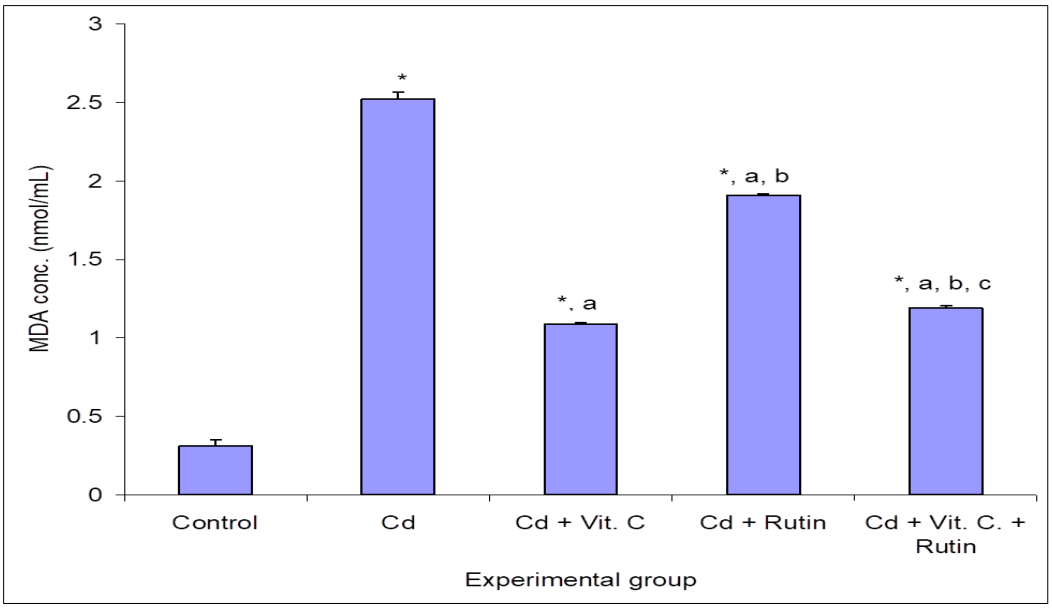
3.4.4. Malondialdehyde concentration

The concentration of MDA for the control, Cd-only, Cd+vit C, Cd+Rutin and Cd+vitC+Rutin groups were 0.31±0.4, 2.52±0.046, 1.09±0.01, 1.91±0.01 and 1.19 ±0.15 respectively. There was a significant increase in the MDA in the Cd-only compared with the control group (P<0.05) but significantly decreased in all treated groups compared with Cd-only group (P<0.05). It was significantly higher in all treated groups than in the control and higher in the Cd+Rutin than in Cd±vit C groups as shown in Fig 4.



Values are expressed as mean + SEM, n= 5; *=p<0.05 vs control; a= p<0.05 vs cd b = p< 0.05 vs cd + vit, c

Figure 3 Comparison of catalase concentration in the different experimental groups



Values are expressed as mean + SEM, n= 5; *=p<0.05 vs control; a= p<0.05 vs cd b = p< 0.05 vs cd + vit, C; c= p< 0.05 vs CD= Rutin

Figure 4 Comparison of catalase concentration in the different experimental groups

4. Discussion

The study evaluated the comparative effects of vit C and Rutin on anxiety-like behaviours and motor co-ordination in Cd-induced neurotoxic mice. The results are discussed.

The light and dark transition box test is based on the natural aversion of mice to brightly illuminated areas and their spontaneous exploratory behavior in response to mild stress like novel environments and light. The reduction in dark box duration and the increase in light box duration in the Cd+Rutin groups indicate that the mice spent less time in the dark and more time in the light suggesting that it is not afraid or anxious as also pointed out by Takao and Miyakawa [36] and Bailey and Crawley, [37]. This implies that Rutin may have an anxiolytic property better than Vitamin C.

The stretch attempt posture was not significantly different among the groups. This fact added to the earlier observation of non-significant differences in light and dark transitions suggests that Cd exposure might not affect anxiety-like behaviors. The observed decrease in the dark box duration and increased duration in the Cd+Rutin group suggests that Rutin may have an anxislytic property.

The elevated plus maze is another apparatus that is used to assess anxiety-like state in lower animals. It is based on the general aversion of rodents to open space. Our results did not show that exposure to Cd significantly affected the indices used for anxiety-like behaviors in this study, (head dips, stretch attend posture, open arm entry frequency and duration as well as the close arm entry frequency and duration). This indicates that Cd exposure may not be associated with anxiety. However, the observed significance decrease in stretch attend posture, and increase in open arm entry frequency in the Cd+vitC+Rutin compared to the Cd-only and Cd+vit C groups suggests a synergistic effect of vit C and Rutin. In anxiety-like or fear states, the animals have increased duration and frequency in close arm entry due to aversion for open space which was not the case here [33].

The beam walking test is a test for motor coordination [38]. The significant increase in foot slips in the Cd-only group compared with the control suggests that exposure to Cd may be associated with motor incoordination. The significant reduction in foot slips in the Cd+vit C, Cd+Rutin and Cd+vitC+Rutin compared with the Cd-only groups, noted in this study suggests that both vit C and Rutin mitigated this effect (incoordination) non-differentially. In disease states affecting motor coordination, there is increased foot slip [38].

The significantly increased concentration of MDA in the Cadmium-only group compared with the control suggests that Cadmium causes increased lipid peroxidation with subsequent increase in production of reactive oxygen species. This observation is in line with previous studies that show that Cadmium causes increased lipid peroxidation [39,40]. Malondiadehyde is one of the final products of lipid peroxidation [41]. This trend was reversed in all treated groups likely due to the antioxidant nature of vitamin C and Rutin, with vitamin C mitigating the effects better than Rutin.

Our result also suggests that the Cd-induced decrease in catalase activity was mitigated by co-administration with vitamin C or Rutin with vitamin C improving the concentration of catalase better than Rutin.

Cadmium caused a significant decrease in SOD activity in the mice brains which was mitigated with co-treatments with vitamin C, Rutin or both. Vitamin C has a better mitigating effect than Rutin. The decrease is SOD activity noted in the Cd-only group is similar to reports by preventing authors [42].

Glutathione peroxidase, like catalase and SOD is a cellular antioxidant enzyme [43]. Though its activity did not significantly change with exposure to Cd, supplementation with vitamin C and Rutin boosted it activity. This is likely made possible due to their antioxidant nature. Vitamin C demonstrated a better GPx boosting activity than Rutin.

5. Conclusion

We conclude that Cd might not have a significant effect on anxiety-like behaviors but may affect motor coordination which was mitigated non-differentially by supplementation with vitamin C or Rutin. Cd-induced oxidative stress was mitigated by co-administration with vitamin C or Rutin with vitamin C showing a greater effect in that regard than Rutin.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no competing interests.

Statement of ethical approval

The protocol for this study was approved by the Animal Ethics and Research Committee of the Faculty of Basic Medical Sciences, University of Calabar.

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References

[1] World Health Organization. Preventing Disease Through healthy environments. Exposure to Cadmium: a major public health concern. 2019. Available from https://www.who.int/publications/i/it

[2] Pappas RS (2012). Toxic elements in tobacco and cigarette smoke: Inflammation and sensitization. Metalomics 3 (11) 1181-1198.

[3] IPCS (1992). Cadmium. International program of chemical safety (Environmental Health, Criteria 134) WHO, Geneva. Available from https://www.inchem.org/documents/ehc/ehc/ehc/3.

[4] Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A et al. The toxicity of Cadmium and the resulting hazards for human health. Journal of Occupational Medicine and Toxicology. 2006; 1:22 Doi: 10.1186/1745.

[5] Yang S, Chen S, Liang C, Shi Y, Chen Q. Effect of Cadmium exposure on Leydig cells and blood vessels in mouth testes.International Journal of Environmental Research and Public Health. 2021; 19(4) 2416.

[6] Thatcher RW, Lester ML, Mc Alaster R, Horst R. Effects of low levels of Cadmium and lead on cognitive functioning in children. Archives of Environmental Health. 1982; 37(3) 159-66.

[7] Dumpala S, Ramaneswari K, Christada V. Cadmium induced neurotoxity. In Jha, A.K, Kumar (eds) Cadmium toxicity mitigation. Springer, Cham.2024

[8] Lopez F, Figueroa S, Oset-GasqueMJ, Gonzales MP. Apoptosis and necrosis: two distruct events induced by Cadmium in cortical neurons in culture: British Journal of Pharmacology. 2023; 138(5) 901-911.

[9] Monroe RK, Halorsen SW. Cadmium blocks receptor mediated. JAK/STAT signaling in neurons by oxidative stress. Free Radical Biology and Medicine. 2006; 41(3): 493-502.

[10] Qu F, Zhang W. Cadmium exposure: mechanisms and pathways of toxicity and implications for human health. Toxics. 2024, 12(8)388

[11] Souzo-Arroyo V, Fabian JJ, Bucio-Ortiz L, Mirando-Labra RU, Gomez-Quiroz, LE, Gutierrez-Ruiz . The mechanism of the Cadmium induced toxicity and cellular response in the liver. Toxicity. 2022; 480: 153339 Available from www.sciencediet.com

[12] [12] Combs GF, McClung JP. The vitamins fundamental aspect in nutrition and health. Elsevier Inc, Netherlands. 2017.

[13] Micronutrient Information Center. Vitamin C. Linus Pauling Institute, Corvallis. 2017.

[14] Clark S. Comparing milk, human, cow, goat and commercial infant formula. Washington State University, Washington.2007.

[15] Padayatty SJ, Kutz A, Wany Y, Eck P, Kwon O, Lee J, Chan S, Corpse C, Dutta A, Dutta S.K, Levine. Vitamin C as an antioxidant: evaluation of its role in disease prevention. Journal of the American College of Nutrition. 2003; 22(1) 18-35.

[16] Pawlowska E, Szczepanska J, Blasiak J. Pro- and antioxidant effects of vitamin C in cancer in correspondence to its dietary and pharmacological concentrations. Oxidative Medicine and Cellular Longevity. 2019; Vol 2019 (1) 7286737 Available from https:doi.org/10.1155/2019/7286737

[17] Skrzydlewska E, Gegotek A. Ascorbic acid as antioxidant. In Litwack G (ed) vitamins and Hormones. Elsevier Inc, Netherlands.2022

[18] Pehlivan FE. Vitamin C: an antioxidant agent. In Hamza, A.H (ed) Vitamin C. IntechOpen Limited, London. 2017

[19] Krishman UM, Sivananthan B. Rutin and its application to amyotropic lateral sclerosis. In Martin CR, Patel VB, Preedy VR (eds). Treatment, Neutriceuticals, supplements and herbal medicine in neurological disorders. Elsevier, Netherlands. 2023

[20] Oboh G, Ademosun AO, Ayeni PO, Omojokun OS, Bello F. Comparative effect of quercetin and rutin on amylase, x-glucosidase, and some pro-oxidants induced lipid peroxidation in rat pancreas. Comparative Chemical Pathology. 2015, 24:1103-1110.

[21] Patel P, Patel DK. The beneficial role of Rutin, a naturally occurring flavonoid in health promotion and disease prevention: a systemic review and update. In Watson RR and Preedy VR (eds) Bioactive food as dietary interventions for arthritis and related inflammatory diseases. Elsevier, Netherlands. 2019

[22] Saluja AK, Garneshpurkar A. The pharmacological potential of Rutin. Saudi Pharmaceutical Journal. 2016; 25(2) 149-164.

[23] Gillen G, Rubio KB. Treatment of cognitive-perceptual deficits. In: Gillen G (ed) stroke rehabilitation. Elsevier, Netherlands . 2016

[24] Huang L, Xiao D, SunH, Qu Y, Su X.Behavioral tests for evaluating the characteristics of brain diseases in rodent models: optimal choices for improved outcomes (Review). Molecular Medicine Reports. 2023; 25(5).

[25] Davies KG, Etagha N, Aribo E, AntaiAB, Osim EE. Effects of Artemether and Artesunate on social behavior and pain perception. Research in Neuroscience. 2013; 2(3) 31-38

[26] Marklund S. Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice. Mutagen Research. 1985; 148 (1-2) 129-34

[27] Aebi H. Catalase in vitro. Methods Enzymology. 1984; 105:121-126

[28] Rice-Evans C, Miller N, Paganda G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1987; 2:152-159

[29] Jiang W, Feng L, Liu Y, Jiang J, Hu K, Li S, Zhon X. Lipid peroxidation, protein oxidant and antioxidant status of muscle, intestine and hepatopancrease for juvenile jian carp (Cyprinus Carpio var jian) fed graded levels of myo-inositol. Food Chemistry. 1992; 120 (3)692-697

[30] Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for that anxiolytic effects of benzodiazepines. Pharmacology and Biochemistry in Behavior. 1980; 13(2)167-170.

[31] Serchov T. van Calker D, Biber K. Light-dark transition test to assess anxiety-like behavior in mice. Bio-protocol. 2018; 6 (19); dio:10.21769/BioProtoc-1957.

[32] Costall B, Jones BJ, Kelly ME, Naylor RJ, Tomkins DM. Exploration of a mice as a model of anxiety in a black and white test box-validation as a model of anxiety. Pharmacology and Biochemistry in Behavior. 1989; 32 (3): 777-785

[33] Pellow S, Chopin P, File SE, Briley M. Validation of open: Closed arm entries in an elevated plus maze as a measure of anxiety in the rat. Journal of Neuroscience Methods. 1985; 14(3):149-67.

[34] Lister RG. The use of a plus maze to measure anxiety in the mouse. Psychopharmacology (Berl). 1987; 92 (2) 180-185.

[35] Trullas R, Skolnick P. Deficiencies in fear motivated behaviors among inbred mouse strains. Psychopharmacology. 1993; 111:323-331.

[36] Takao K, Miyakawa T. Light/dark transition test for mice. Journal of Visualized Experiments. 2006; 13(1)104. Doi 10.3791/104

[37] Bailey KR, Crawley JN. Anxiety related behaviors in mice. In: Buccafusco JJ (ed), Methods of Behavior Analysis in Neuroscience. CRC Press/Taylor and Francis, Boca Raton (FL). 2009

[38] Luong T, Carlisle HJ, Southwell A, Patterson PH. Assessment of motor balance and coordination in mice using the balance bean. Journal of Visualized Experiments. 2011; 49:2376 doi:10.3791/2376

[39] aAribo EO, Nwangwa JN, Udokang NE, Aribo RE. Cadmium-induced nitric oxide synthase up-regulation in Wister rats: Ameliorative effect of STC30, a polyherbal remedy. I0SR Journal of Environmental Science, Toxicology and Food Technology. 2024; 18(8) 11-15.

[40] bAribo EO, Nwangwa JN, Udefa AL, Antai AB. A polyherbal remedy, purxcel, improves Cadmium-induced male reproductive impairment and testicular antioxidant status in Wistar rats. Nigerian Journal of Physiological Sciences. 2024; 39(1)125-135.

[41] Rio DD, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as a toxic molecule and biological marker of oxidation stress. Nutrition, Metabolism and Cardiovascular Diseases. 2005; 15(4)316-328

[42] Nwangwa JN, Aribo EO, Okpoene AI. Mitigating cadmium-induced reproductive toxicity in male Wistar rats: protective role of STC 30, a polyherbal formulation. Journal of Complementary Medical Research. 2024; 25 (10), 40-55.

[43] Ighadaro OM, Akinloye AO. First line defense antioxidants: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx): their fundamental role in the entire antioxidant defense grid. Alexandria Journal of Medicine. 2018; 54(4):287-293