

Original Research Article

Cadmium-induced Up-regulation of Nitric Oxide Synthase Activity in Rats' Testes: Ameliorative Effect of PurXcel

ABSTRACT

Background: Cadmium is a common environmental pollutant and induces testicular dysfunction via mechanisms which include oxidative stress linked with inflammation. PurXcel is a polyherbal remedy said to be rich in antioxidants. The action of Cadmium on nitric oxide homeostasis and possible effect of the polyherbal on any functional aberration from this are not known and hence this study.

Methods: Twenty male Wistar rats were randomly assigned into control, Cadmium-only, PurXcel-only and Cadmium+PurXcel groups of five rats each. Duration of daily administration was 28 days after which animals were sacrificed and their testes dissected out for determination of necessary parameters.

Results: The results showed a significant increase ($p \leq 0.05$) in the testicular activity of Nitric Oxide synthase in the Cadmium-only group compared with the control but which was significantly lower in the PurXcel-only and Cadmium+PurXcel groups than in the Cadmium-only group ($p \leq 0.05$). The concentration of testicular nitric oxide was significantly increased in the Cadmium-only group compared with the control ($p \leq 0.05$) but significantly lower in the Cadmium+PurXcel and PurXcel-only groups than in the Cadmium-only group ($p \leq 0.05$). The levels were significantly higher in the Cadmium+PurXcel than in the PurXcel-only groups ($p \leq 0.05$).

Conclusion: In conclusion, PurXcel administration ameliorates Cadmium-induced up-regulation of Nitric Oxide Synthase activity and the resultant increase in the concentration of nitric oxide in rats testes. Therefore, dysregulation of Nitric Oxide homeostasis may play an important role in Cadmium-induced cytotoxicity

Keywords: Cadmium, PurXcel, up-regulation, Nitric Oxide Synthase, Nitric Oxide

1. INTRODUCTION

Cadmium is a major environmental pollutant and finds itself in a wide range of applications from the industry to Agriculture [1,2]. It is found in battery, plastics, paint, antirust coatings, glass, fertilizer, solar panels, semiconductors, QLED television, photocopier drums, cigarette, etc [3,4].

From these sources, Cadmium finds itself into the environment and **contaminates** it. Workers involved in industries that manufacture the above products or who use or recycle these products are at high risk of exposure to Cadmium [1]. Exposure to Cadmium occurs **primarily** by ingestion of contaminated water sources and food as well as by inhalation. It is also easily transferred from soil to plants consumed by animals and humans [5].

The negative effects of Cadmium on human health have been well documented. It is known to cause kidney damage [6], female reproductive impairment [7], dyslipidemia [8] as well as male reproductive toxicity [9,10].

The negative effect of Cadmium on tissues has been partly blamed on oxidative stress, an imbalance in redox status of cells [11,12]. This assertion is supported by the improvements observed in function and redox status following treatment with various antioxidants on Cadmium-induced **cytotoxicity** [9,10,13].

Nitric Oxide Synthase (NOSs) are oxidoreductase heme proteins that catalyze the chemical reaction in which nitric oxide is formed from L-arginine and oxygen. The neuronal and endothelial isoforms of this enzymes are of low output and constitutively expressed in neuronal and endothelial cells causing the synthesis of nitric oxide in pulsatile manner [14]. The inducible isoform (iNOS) of the enzyme can be expressed in almost any cell types in response to polysaccharides, cytokines or other agents [15]. Inducible nitric oxide synthase is therefore upregulated in inflammation, septic conditions, cancers and oxidative stress and results in increased production of nitric oxide [16,17].

Nitric oxide is a highly reactive signaling molecule capable of permeating cellular membranes and in normal conditions involved in the regulation of several physiological events like vasodilatation, neurotransmission and apoptosis [18]. Its physiological role is also seen in inhibition of DNA synthesis and mutagenesis, antiviral, antimicrobial, cytoprotection, vascular smooth muscle relaxation and regulation of hypothalamo-pituitary-gonadal axis [19].

The production of nitric oxide and its conversion to reactive nitrogen species modulate the balance of its in vivo actions in cytoprotection, or cytotoxicity [20]. Its concentration is therefore increased following upregulation of nitric oxide synthase activity [16,17].

There is a growing interest in the development and usage of polyherbal remedies rich in antioxidants to solve health problems as they are said to be cheaper and have fewer side effects [21,22]. PurXcel is an example of such remedies said to have high antioxidant contents.

PurXcel is a proprietary product of LivePure, Frisco, Texas, USA. It is said to contain about 18 complimentary ingredients like glutathione, aloe acemannan, superoxide dismutase, vitamin C, selenium, alpha lipoic acid, Broccoli, turmeric, blueberry, schizandra, grape seeds, pomegranate and black pepper extract [23]. It has multiple claims of health benefits like hepatoprotection, immune-boosting, improvement of redox status etc [23], most of which have not been authenticated scientifically.

Since the cytotoxicity of Cadmium is partly attributed to oxidative stress, it became necessary to assess the effect of this polyherbal remedy on Cadmium-induced dysregulation of nitric oxide synthase activity which would also shed more light on the mechanisms of tissue injury by Cadmium.

2. MATERIALS AND METHODS

2.1 Chemicals:

Preparation of stock solution of Cadmium: This was made by dissolving 50mg of Cadmium Chloride, CdCl₂, (Sigma-Aldrich, Chemical Company, St Louis, MO, USA) in 50ml of distilled water.

Preparation of stock solution of PurXcel: The content of one capsule (435mg) of PurXcel (Live Pure, Frisco, Texas, USA) purchased from Puregen African Nigeria Limited, Lagos, Nigeria was dissolved in 200ml of distilled water.

2.2 Acute toxicity study: The LD50 of Cadmium was determined using Lorke's method [24] and as a follow-up using the up-and-down method as described by Erhirhie *et al* [25]

2.3 Laboratory animals: Twenty adult male Wistar rats were used for this study. They were housed in metallic cages in the animal house of the Department of Physiology, University of Calabar under standard sanitary conditions. They were given free access to food and water.

2.4 Experimental design: Twenty male wistar rats were randomly divided into four groups of five rats each. Group one served as the control, group two was the Cadmium-only group, group

three was PurXcel-only while group four was Cadmium+PurXcel group. Cadmium Chloride was administered at a dose of 5mg/kg [26,27] while PurXcel was administered at an oral dose of 38.4mg/kg based on the computation of effective dose described by Nair and Jacob [28]. The duration of daily administration of the drugs was 28 days. The control group was given 0.5ml of the vehicle daily.

2.5 Sample collection: At the end of the treatment period, animals were anaesthetized with pentobarbital (60mg/kg) and blood samples collected from them via cardiac puncture after which animals were sacrificed and their testes harvested for determination of relevant parameters.

2.6 Preparation of testicular homogenate: The left testes of each rat was homogenized separately in 50µl Tris-HCl buffer (pH 7.4) containing 1.15% KCl to prepare a 20% (1/5w/v) tissue homogenate using Potter Elvehjem homogenizer (BEE International, Apion Company, USA). It was then centrifuged at 10000g for 10minutes in a cold centrifuge. The supernatant were obtained and used for determination of necessary testicular parameters.

2.7 Determination of testicular nitric oxide concentration: This was done colorimetrically in testicular homogenate using commercially available kits (BioAssay Systems, Haywood, CA, USA) according to the manufacturer's protocol with the optical density read at 500-570nm.

2.8 Determination of testicular nitric oxide synthase activity: The activity of nitric oxide synthase in the testes was measured by evaluating the conversion of L-[³H] arginine to [³H] citrulline [29] in the homogenate.

2.10 Statistical analysis: Data was expressed as mean±SEM and analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test of least significant difference. Values of $p \leq 0.05$ were considered statistically significant.

3. RESULTS

3.1 Testicular concentration of Nitric oxide (NO)

The concentrations of NO (nmol/100mg tissue) were 31.8±3.193, 83.2±3.420, 22.4±2.073 and 49.8±2.387 for control, Cadmium-only, PurXcel-only and Cadmium+PurXcel groups respectively. There was a significant increase in the levels of NO in the Cadmium-only group compared with the control ($p \leq 0.05$) but significantly lower ($p \leq 0.05$) in the PurXcel-only and Cadmium+PurXcel than in the Cadmium-only groups. It was also significantly lower

($p \leq 0.05$) in the PurXcel-only and higher in the Cadmium+PurXcel than in the control groups ($p \leq 0.05$) as shown in Fig. 1.

3.2 Activity of nitric oxide synthase (NOS)

The testicular activity of NOS (pmol/mg/20 minutes) were 0.5 ± 0.1 , 1.14 ± 0.181 , 0.6 ± 0.158 and 0.72 ± 0.130 for control, Cadmium-only, PurXcel-only and Cadmium+PurXcel groups respectively. Nitric oxide synthase activity was significantly increased in the Cadmium-only group compared with the control ($p \leq 0.05$) but significantly lower in the PurXcel-only and Cadmium+PurXcel groups than in the Cadmium-only ($p \leq 0.05$) as shown in Fig. 2.

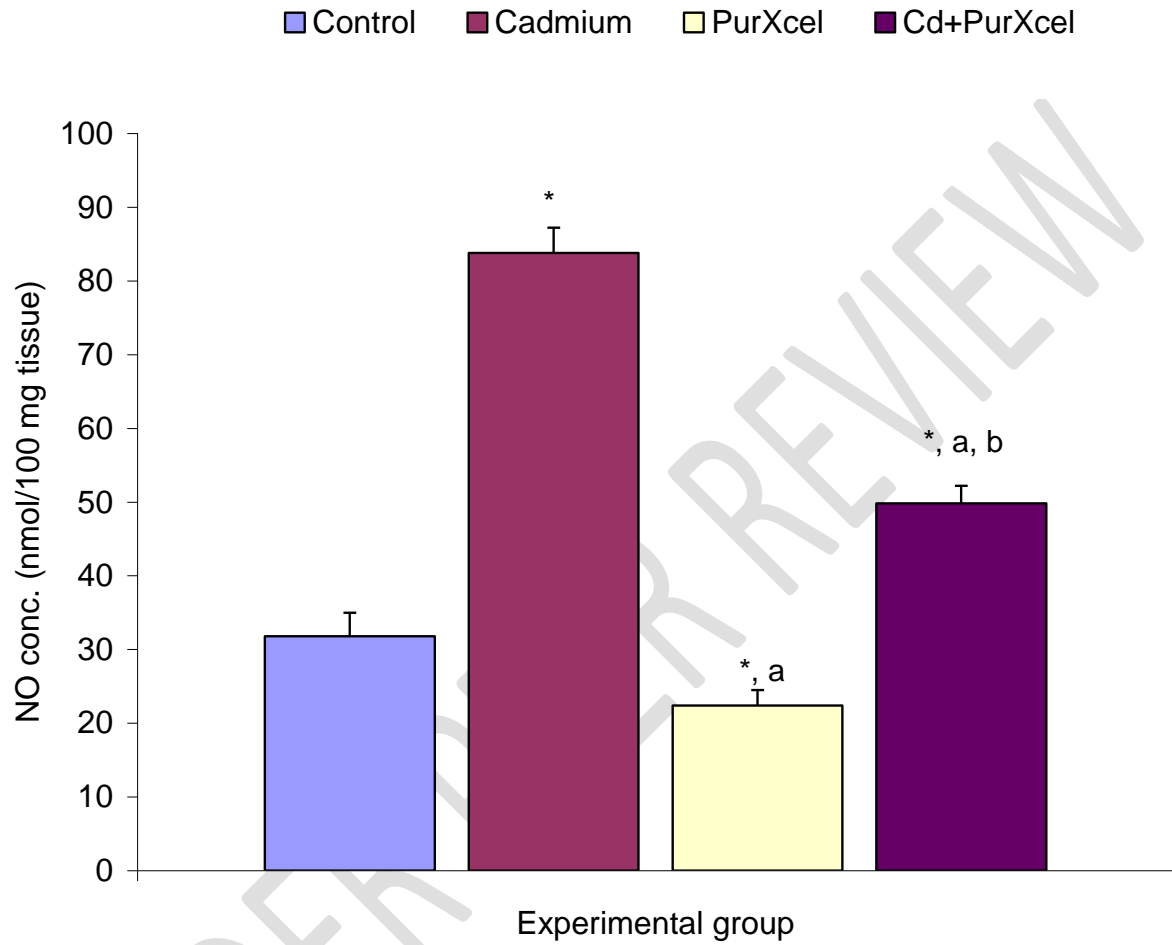


Fig. 1: NO concentration in the different experimental group.

Values are expressed as mean +SEM, n = 5.

* = $p \leq 0.05$ vs Control

a = $p \leq 0.05$ vs Cadmium

b = $p \leq 0.05$ vs PurXcel

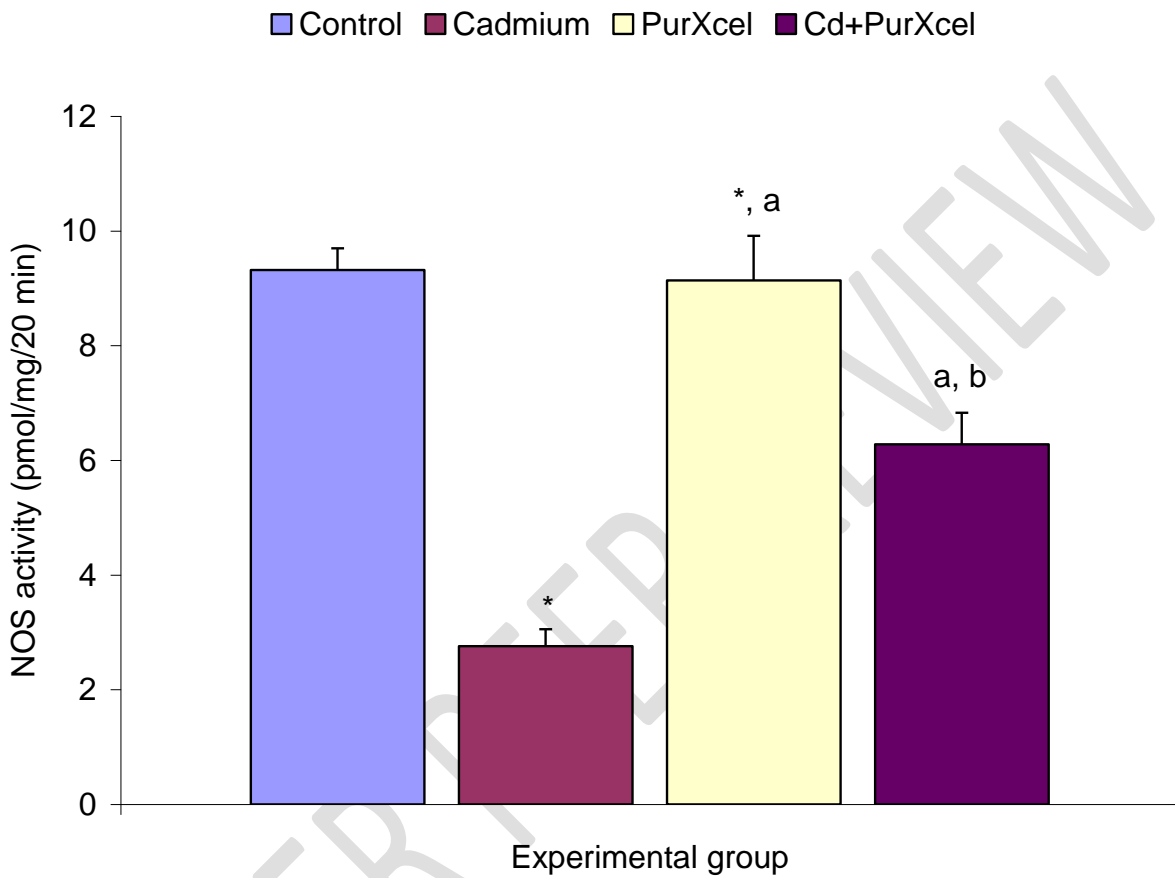


Fig. 2: Nitric oxide synthase activity in the different experimental group.

Values are expressed as mean +SEM, n = 5.

* = $p \leq 0.05$ vs Control

a = $p \leq 0.05$ vs Cadmium

b = $p \leq 0.05$ vs PurXcel

4. DISCUSSION

The activity of nitric oxide synthase and the levels of nitric oxide in testicular tissue following administration of Cadmium Chloride with/without treatment with PurXcel were studied. Our findings are discussed.

From our results, exposure to Cadmium Chloride causes an elevation in the levels of testicular NO. Nitric oxide, a labile molecule usually produced in small amount is an important signaling molecule in several physiological functions in immune responses, blood flow, smooth muscle relaxation and neurotransmission [18,30].

The observed increase in the level of NO might have been due to its over-production stimulated by Cadmium in the testes through a yet-to-be identified mechanism. Though NO at physiological levels is needed for normal body functions [31,32], at high concentrations it is associated with inflammation, sepsis and cytotoxicity [33] due to its oxidation of biomolecules [34]. The elaboration of NO in tissues including the reproduction system noted in this study might therefore at least in part explain a possible mechanism by which Cadmium induces tissue injury [10,35]. This is strengthened by our observation that co-administration of Cadmium with PurXcel which is said to contain potent antioxidants reduced the concentration of NO in the testes .

Though co-administration of Cadmium with PurXcel significantly reduced testicular concentration of NO compared with the control, it was still higher than in the Cadmium-unexposed rats. This suggests that, PurXcel has limited ability to restore NO concentration to its pre-exposure levels. It can also be said from the results that PurXcel administered alone has the potential to reduce the concentration of NO in the testes since its level was lower in the PurXcel-only group compared with the control.

The observed reduction in the Cadmium-associated elevation of NO following co-administration with PurXcel might have been due to the effect of antioxidants said to be present in PurXcel [23].

Cadmium administration resulted in a significant increase in the activity of nitric oxide synthase which was prevented following co-administration with PurXcel. Upregulation of NOS can be triggered by many factors including cytokines, intracellular bacteria, inflammation, septic conditions [16] and oxidative stress [17]. Cadmium administration is also known to induce oxidative stress [9]. Therefore, the upregulation in the activity of NOS observed in this study

might have been due to Cadmium-induced oxidative stress. This is supported by the significant prevention of this dysregulation following co-administration of Cadmium with PurXcel which is said to be rich in antioxidants [23].

5. CONCLUSION

We therefore conclude that exposure to Cadmium causes dysregulation of Nitric Oxide homeostasis in testes of Wistar rats, but which is ameliorated by PurXcel administration. Nitric Oxide Synthase Dysregulation therefore might play critical role in Cadmium-induced cytotoxicity.

ETHICAL APPROVAL: The approval of this study was granted by the Animal and Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar. (Approval No. 256PHY2103).

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- 1.
- 2.
- 3.

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