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Cadmium-Induced Testicular Nitric Oxide Synthase Up-Regulation In Wistar Rats: Ameliorative Effect Of Stc30, A Polyherbal Remedy.

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Abstract
Background: *Male infertility is a growing world-wide problem. There are equally several causes of male infertility ranging from environmental pollutants, drugs and microbials to lifestyle changes. Cadmium is one of such environmental pollutants with adverse effect on testicular function attributed in part to oxidative stress. Development and patronage of polyherbal remedies which are said to be rich in antioxidants and have fewer side effects to treat or prevent health problems are on the increase. Superlife Total Care (STC30) is one of such polyherbal remedies. The effect of Cadmium on testicular nitric oxide homeostasis and the possible effect of STC30 are not known and hence the need for this study.*
Materials and methods: *Twenty mature male Wistar rats were used for this study. They were divided into control, Cadmium-only, STC30-only and Cadmium+STC30 groups of 5 rats each. Rats were orally administered Cadmium Chloride and/ or STC30 for 28 days. The rats were then sacrificed and their testes harvested for determination of necessary parameters.*
Results: *Our results show a significantly increased level of nitric oxide in the Cadmium-only group compared with the control ($p<0.05$) but a significantly lower level in the STC30-only and Cadmium+STC30 groups than in the Cadmium-only group ($p<0.05$). Nitric oxide level was significantly lower in the STC30-only though higher in the Cadmium+STC30 groups than in the control ($p<0.05$). Nitric oxide synthase activity in the testes was significantly increased in the Cadmium-only group compared with the control ($p<0.05$) but significantly lower in the STC30-only and in the Cadmium+STC30 groups than in the Cadmium-only group ($p<0.05$).*
Conclusion: *In conclusion, Cadmium administration causes upregulation of testicular nitric oxide synthase activity and consequent increase in the level of nitric oxide which was ameliorated following treatment with STC30.*
Keywords*: Cadmium, STC30, Up-regulation, nitric oxide, nitric oxide synthase*

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I. Introduction

Infertility is a global challenge. The World Health Organization[1] reported that 17.5% (about 1 in 6) of the adult population worldwide experience infertility which has social, economic and emotional consequences[2]. A research by Huang et al[3] indicates that there is a steady increase in the global burden of male factor contribution to infertility over the past 30 years. Studies indicate that male factors alone account for about 20 to 30% of infertility cases while around 50% of couples experience infertility due to male factor . A similar observation on the rise of male factor infertility is also reported here in Nigeria[5,6].

Although some of the causes of male infertility remain idiopathic[7], obesity, nutritional, lifestyle changes, microbials[8,9,10] and environmental contaminants and endocrine disruptors[11,12] have played critical roles in male factor infertility. One of such environmental pollutants and endocrine disruptors is Cadmium[13,14].

Cadmium is a toxic metal that finds its application in many products including electronics/electronical parts, coating materials, plastics, ceramics, battery, fireworks, refined petroleum products and fertilizers[14,15]. Exposure to Cadmium is through inhalation, drinking water and eating food grown in contaminated soils[1] . Cigarette smoke and exhaust fumes are also common sources of Cadmium exposure[17].

Cadmium exposure is known to have deleterious effects on various organs and systems including the respiratory, renal, nervous, hepatic, hematopoietic and reproductive systems[18,19]. Cadmium is an endocrine disruptor[13]. Males exposed to Cadmium have been reported to have reproductive impairment like defective steriodogenesis, semen quality, associated with teratozotospermia and infertility[20,21,22,23].

Nitric oxide is a highly reactive signaling molecule which under normal concentrations and conditions is involved in the regulation of several physiological events like vasodilatation, neurotransmission and

apoptosis[24]. Its physiological role is also seen in its antiviral, antimicrobial, cytoprotection and vascular smooth muscle relaxation effects as well as regulation of hypothalamo-pituitary-gonadal axis[25]. Achieving a physiological balance between NO and O_2- (nitroso-redox balance) is critical to the regulation of physiological functions[26]. Its concentration is therefore increased following upregulation of nitric oxide synthase activity[27,28]. Previous studies have reported an up-regulation of Nitric Oxide Synthase activity and an increase in nitric oxide concentration following exposure to Cadmium[29].

Nitric oxide synthases (NOSs) are oxidoreductase heme-containig proteins that catalyze the formation of nitric oxide from L-arginine and oxygen. The endothelial and neuronal isoforms of this enzymes are normally produced in low quantity and constitutively expressed in neuronal and endothelial cells causing the synthesis of nitric oxide in a pulsatile manner[30]. The inducible isoform (iNOS) of the enzyme can be expressed in almost any cell types in response to cytokines, polysaccharides or other agents[31]. Nitric oxide synthase is therefore upregulated in inflammation, septic conditions, cancers and oxidative stress resulting in increased elaboration of nitric oxide[27,28].

The mechanisms by which Cadmium induces cytotoxicity are not fully known though this is in part attributed to oxidative stress[20,21]. This is supported by the ability of different antioxidants in different studies to ameliorate Cadmium-induced toxicity in tissues[5,21,32]. Also, the role of antioxidants in ameliorating cytotoxicities is well documented[33]. Recently, much attention has been directed towards the development and usage of polyherbal remedies said to be rich in antioxidants and which are believed to have fewer side effects to prevent or treat medical conditions[34]. Superlife Total Care (STC30) is one of such polyherbals.

Superlife Total Care (STC30) is a proprietary polyherbal remedy made by Superlife World, Kualar Lumpur Malaysia[35]. It is said to contain blackcurrant juice rich in polyphenolic substances, anthocyanins, anti-oxidants, gamma linoleic acid and vitamin C, substances known to have antioxidant effects[35,36,37]. It also contains bilberry extract which is said to be hepatoprotective and nitric oxide homeostasis maintainer with strong antioxidative effect[38]. Superlife Total Care (STC30) improves renal and hepatic impairments in hepato- and renal toxicities caused by CCl_4[39,40].

Limited studies have been conducted on STC30 as cited above. There is however paucity of information on its effect on nitric oxide homeostasis following Cadmium administration which could throw more light on the pathophysiology of Cadmium-induced cyttotoxicities and hence this study.

II. Materials And Methods

Ethical approval: The ethical approval for this study was given by the Animal Research and Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Calabar (Approval No. 256PHY2103).

Chemicals:

Preparation of stock solution of Cadmium: Cadmium Chloride, $CdCl_2$, (Sigma-Aldriech, Chemical Company, St Louis, MO, USA) was dissolved in 50ml of distilled water to prepare a stock solution of Cadmium Chloride.

Preparation of stock solution of STC30: To prepare the stock solution of STC30, the content of one capsule (1500mg) was dissolved in 200ml of distilled water.

Acute toxicity study: The LD50 of Cadmium was determined using Lorke's method[41] and using the up-and-down method as described by Erhirhie *et al*.[42] as a follow-up.

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

Experimental design: The twenty male Wistar rats were randomly divided into 4 groups of 5 rats each. Group 1 served as the control, group 2 was the Cadmium-only, group 3 as the STC30-only and group 4 as the Cadmium+STC30 group. Cadmium Chloride was given at a dose of 5mg/kg[43] and STC30 at a dose of 132.7mg/kg, its effective dose calculated from its therapeutic dose[44]. The duration of administration by daily gavaging was 28 days. The animals were weighed regularly and the amount of their drugs adjusted accordingly. The control group was given 0.5ml of the vehicle (distilled water) daily.

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 following which animals were sacrificed and their testes excised for determination of relevant parameters.

Preparation of testicular homogenate: The left testes of each rat was excised and 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 for 10minules at 10000g in a cold centrifuge. The supernatants were obtained and used for assessment of necessary parameters in the tested.

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

Determination of testicular nitric oxide synthase activity: The activity of testicular nitric oxide synthase was measured by evaluating the conversion of L-[^{3}H] arginine to [^{3}H] citrulline[45] in the homogenate.

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 $p<0.05$ were considered statistically significant.

III. Results

Testicular concentration of nitric oxide (NO)

The mean¥SEM concentrations of NO (nmol/100mg tissue) for the control was 31.8±3.193 and for the Cadmium-only group it was 83.2±3.420. For the STC30-only group it was 22.4±2.073 while it was 49.8±2.387 for the Cadmium+STC30 group. The testicular level of NO in the Cadmium-only group was significantly increased compared with the control ($p<0.05$) but significantly lower ($p<0.05$) in the STC30-only and Cadmium+STC30 than in the Cadmium-only groups. It was also significantly lower ($p<0.05$) in the STC30-only and higher in the Cadmium+STC30 than in the control groups ($p<0.05$) as shown in Fig. 1.

Activity of nitric oxide synthase (NOS)

The mean¥SEM testicular activity of NOS (pmol/mg/20 minutes) for control was 0.5 ± 0.1 and for Cadmium-only group it was 1.14±0.181. It was 0.6±0.158 for the STC3-only group and 0.72±0.130 for the Cadmium+STC30 group. Nitric oxide synthase activity was significantly increased in the Cadmium-only group compared with the control ($p<0.05$) but significantly lower in the STC30-only and Cadmium+STC30 groups than in the Cadmium-only ($p<0.05$) as shown in Fig. 2.

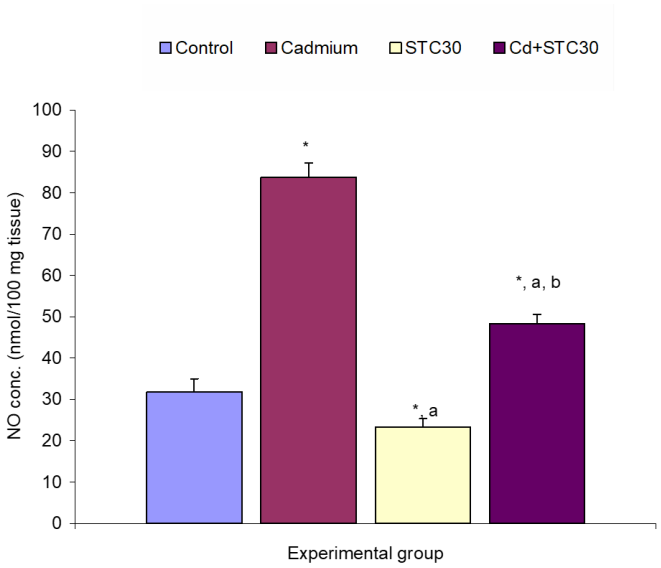

Fig. 1: NO concentration in the different experimental group.

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs Control
a = p<0.05 vs Cadmium
b = p<0.05 vs STC30

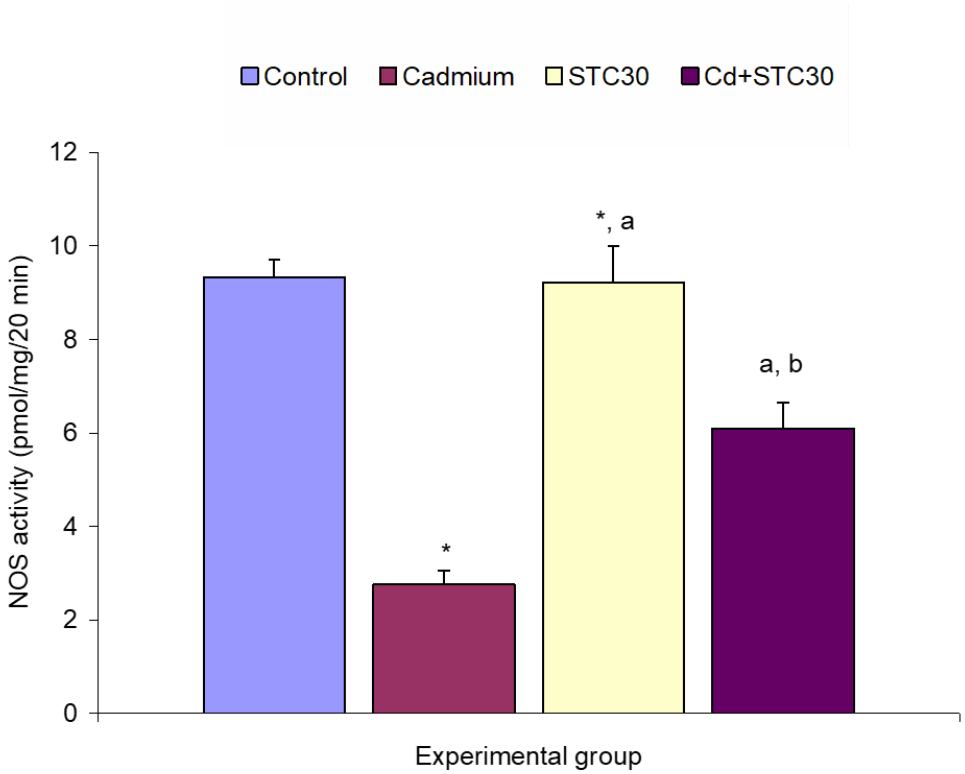

Fig. 2: Nitric oxide synthase activity in the different experimental group.

Values are expressed as mean +SEM, n = 5.
* = p<0.05 vs Control
a = p<0.05 vs Cadmium
b = p<0.05 vs STC30

IV. 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 STC30 were studied. Our findings are discussed.

We noted an elevation in the level of testicular concentration of NO following exposure to Cadmium Chloride. Nitric oxide molecules usually produced in small amounts are important signaling agents in physiological functions in immune responses, blood vessel dilatation, smooth muscle relaxation and neurotransmission in normal concentration[24].

The increase in the level of NO seen in this study 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[46], at high concentrations it is associated with inflammation, sepsis and cytotoxicity[47] due to its oxidative effect on biomolecules[48]. Therefore, its production by effector cells and

conversion to reactive nitrogen species modulate the balance of its in vivo actions in cytoprotection or cytotoxicity[26]. The elaboration of NO in tissues including the reproduction system might therefore at least in part be used to explain the damaging effects of Cadmium on tisseus[21,49]. This assertion is strengthened by our observation that administering Cadmium in combination with STC30 which is said to contain potent antioxidants was able to reduce the concentration of NO in the testes .

Alhough the level of testicular NO was reduced following co-administation of Cadmium with STC30 when compared with the Cadmium-only group, it was still higher than in the control or Cadmium-unexposed rats. This suggests that, STC30 has limited ability to restore NO concentration to its pre- or non-exposure levels. The significantly lower levels of NO in the STC30-only group compared with the control suggests that STC30 given alone has the potential to reduce the concentration of NO in the testes. This implies that STC30 could be used against medical conditions that are caused by or elaborate nitric oxide levels in tissues. The observed reduction in the Cadmium-associated elevation in NO levels following co-administration with STC30 might have been due to the effect of antioxidants said to be present in STC30[35].

From this study, Cadmium administration caused a significant increase in the activity of nitric oxide synthase which was prevented or reduced by co-administration with STC30. Upregulation of NOS can be triggered by many factors including cytokines, intracellular bacteria, inflammation, septic conditions[27] and oxidative stress[28]. Cadmium administration is known to induce oxidative stress[5]. Therefore, the upregulation in the acivity of NOS observed in this study might have been due to Cadmium-induced oxidative stress. This is supported by the observed amelioration of this dysregulation following co-administration of Cadmium with STC30 which is said to be rich in antioxidants[35]. It is therefore possible that the dysregulation of nitric oxide synthase activity in tissues could have played critical role in the pathogenesis of Cadmium-induced cytotoxicities.

V. Conclusion

In conclusion, exposure to Cadmium upregulates nitric oxide synthase activity in the rat testes, but this up-regulation and the consequent elaboration of nitric oxide levels were ameliorated by administration of STC30. Therefore, nitric oxide may play a critical role in Cadmium-induced cytotoxicities.

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