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 ($p \leq 0.05$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 ($p \leq 0.0$).

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 cytotoxiaties.

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, $CdCl_2$, (Sigma-Aldriech, 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 metalic 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 $L^{-}[^{3}H]$ arginine to $[^{3}H]$ 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 ($p<0.05$) 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.

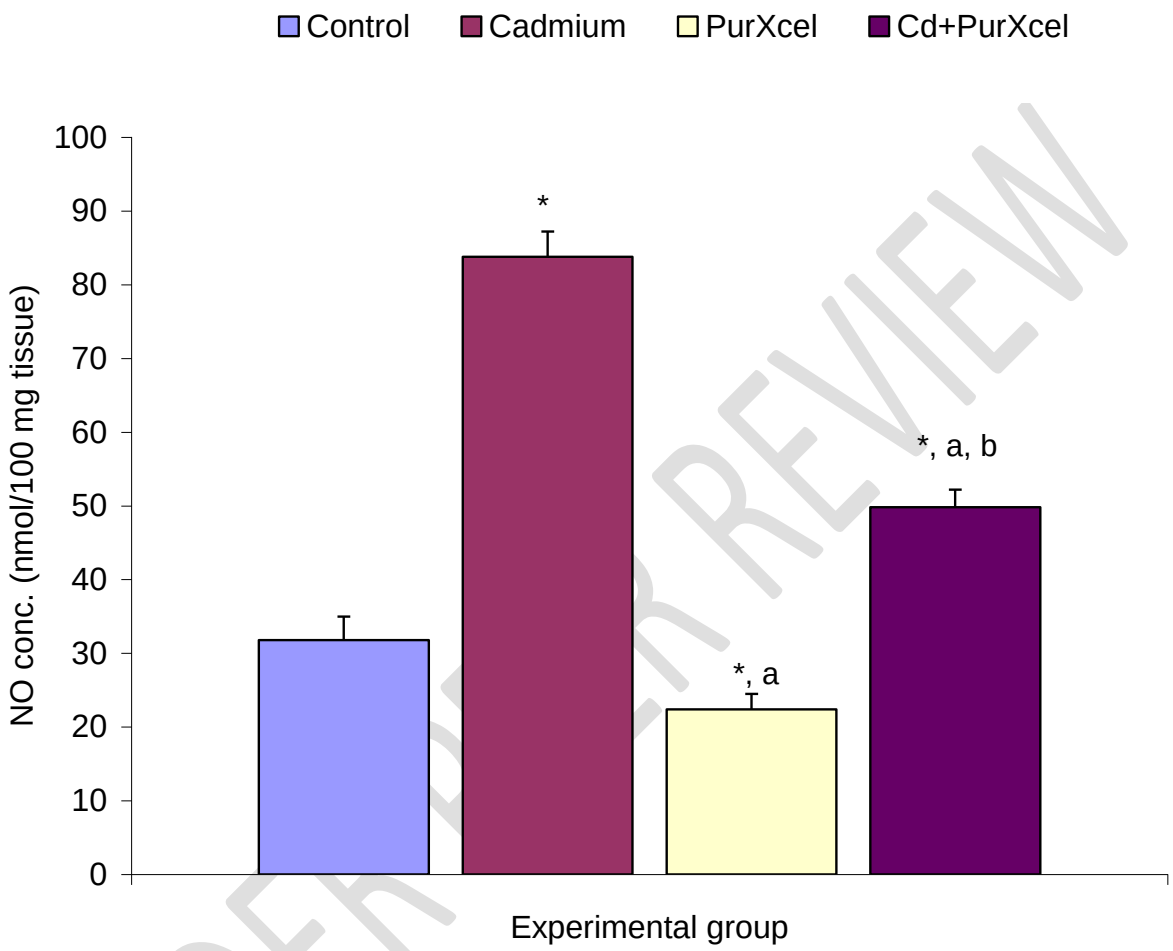

Fig. 1: NO concentration in the different experimental group.

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

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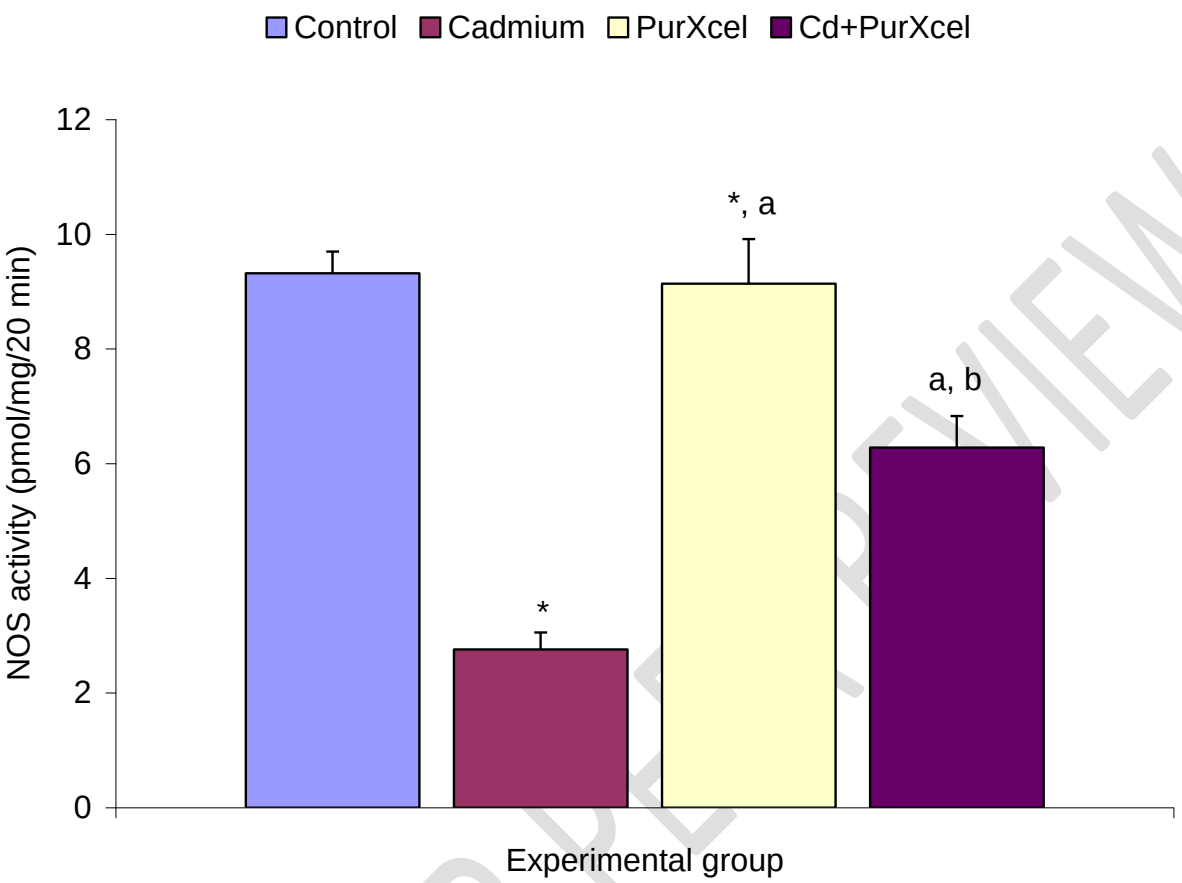

Fig. 2: Nitric oxide synthase activity in the different experimental group.

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

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).

REFERENCES

1. Genchi G, Sinicroppi MS, Lauria G, Carocci A and Catalano A. The effects of Cadmium toxicity. International Journal of Environmental Research and Public Health. 2020; 17(1)3780. Doi:10.3390/ijerph.17123782

2. World Health Organization. Preventing disease through healthy environments-Exposure to Cadmium: A major public Health concern. 2019. http: www.who.

3. Scoullos M.J., Vonkeman G.H., Thorten L. and Mackuch Z. Mercury, Cadmium, Lead: Handbook for Sustainable Heavy Metals Policy and Regulation. Springer. New York. 2001.

4. Unsal V, Dalkiran T, Cicek M, Kolukc U. The role of natural antioxidants against reactive oxygen species produced by Cadmium-toxicity: a review. Advanced Pharmaceutical Bulletin. 2020: 10(2)184-202

5. Mead MN. Cadmium confusion: do consumers need protection? Environmental Health Perspective. 2010. 118(12):A528-A534. https://doi.org/10.1289/who.118-0528

6. Jarup L, Berglund M, Elinder CG, Nordberg G and Varter M. Health effect of Cadmium exposure- a review of the literature and a risk estimate. Scandinavian Journal of Work Environment and Health. 1998. 24(1):1-51

7. Piasek M and Laskey JW. Effects of in vitro Cadmium exposure on ovarian steroidogenesis in rats. Journal of Applied Toxicology. 1999: 19(3):212-217

8. Zhou Z, Lu Y, Pi H, Gao P, Li M, Zhang L, Pei L, Liu L, Zhoa Q, Qin Q, Chen Y, Jiang Y, Zhang F and Yu Z. Cadmium exposure is associated with the prevalence of dyslipidemia. Cellular Physiology and Biochemistry. 2016; 40:633-643.

9. Abarikwu SO, Olufemi PD, Lawrence CJ, Wekere FC and Barikama AM. Rutin, an antioxidant flavonoid induces glutathione peroxidase activity to protect against ethanol effects in Cadmium-induced oxidative stress in the testes of adult rats. Andrologia. 2016; 49:47

10. El-Newesky MS, El-Madawy ZK and El-Sayed YS. Therapeutic effects of date palm (phoenix dactilifera L) Polten extract on Cadmium-induced testicular toxicity. Andrologia. 2012; 1-10.doi:10.1111/and.12025

11. El-Demerdash FM, Yousef MI, Kedwany FS and Baghdadi HH. Cadmium-induced changes in lipid peroxidation, blood hematology, Biochemical parameters and semen quality of male rats: protective role of vitamin E and beta carotene. Food and Chemical Toxicology. 2004; 49:845-854.

12. El-Sayed A, Salem SM, El-Garhy AA , Rahman ZA and Kandil AM. Protective effect of Zinc against Cadmium toxicity on pregnant rats and their fetuses at morphological, physiological and molecular level. African Journal of Biotechnology. 2013; 12(16):2110-2119.

13. Olaniyan OT, Ojewale AO, Eweoya OO, Adedeyia AA, Adeoye AO, Okeniran OS : Modulatory role of vitamin E on proton pump (ATPase) activity of Cadmium chloride-induced testicular damage in Westar Rats. Biomedical Research International. 2021: https://doi.10.1155/2021/4615384.

14. Fostermann U and Sessa WC. Nitric oxide synthase: Regulation and Function. European Heart Journal, 2012; 33(7):821-831
https://doi.org/10.1093/euroheartj/ehr304

15. Siervo M, Stephan BC, Feelisch M, Black LJ. Measurement of in vivo nitric oxide synthesis in humans using stable isotope method: a systematic review. Free Radical Biology in Medicine. 2011; 51:795-804.

16. Wong JM and Billar TR. Regulation and Function of inducible nitric oxide synthase during sepsis and acute inflammation. Advanced Pharmacology. 1995; 34:155-170

17. Pautz A, Art J, Hahn N, Nowag S, Voss C and Kleinert H. Regulation of expression of inducible nitric oxide synthase. Nitric Oxide. 2010; 23: 75-93. Doi:10.1016/j.niox.2010.04.007

18. Gow AJ, Farkouh-Karoleski C, Munson D, Posencheg MA and Ischiropoulos H. Biological significance of nitric oxide-mediated protein modification. American Journal of Physiol Lung Cell Mol Physiol. 2004,; 287(2):L262-8 Doi:10.1152/ajplung.00295.2003

19. Dexit VD and Parwizi N. Nitric oxide and the control of reproduction. Animal Reproduction Journal. 2021; 65(1-2)1-16. https://doi.org/10.1016/S0378-4320(00)0224-4

20. Velayutham M and Zweier JM. Nitric oxide signaling in Biology. Messenger. 2013; 2:1-18. www.biorx1v.org

21. Ogbonnia SO, Mbaka GO, Igbokwe NH, Anyika EN, Alli P and Nwakakwa N. Antimicrobial evaluation, acute and subchorionic toxicity studies of Leone Bitters, a Nigerian polyherbal formulation, in rodents. Agriculture and Biology Journal of North America. 2010; 1(3)366-376.

22. Rana SV. Metals and apoptosis: recent developments. Journal of Trace Elementary Medical Biology. 2010; 22:262-284

23. www.livepure.com

24. Lorke DA. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983; 54:275-287

25. Erhirhie EO, Ihekwereme CP and Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdisciplinary Toxicology. 2018; 11(1):5-12.

26. Da-Costa R; Botana D, Pineros, Proverbio F and Marin R. plasma membrane Ca^{2+}-ATpase and axonemal dynein-ATpase of human spermatozoa. Andrologia. 2016; 48(4)464-9.

27. Nna VU, Ujah GA, Mohamed M, Etim KB, Igba BO, Augustine ER et al. Cadmium chloride-induced testicular toxicity in male wister rats: Prophylactic effect of quercetin and assessment of testicular recovery following Cadmium chloride withdrawal. Biomedicine and Pharma Therapy. 2017; 94:109123

28. Nair AB and Jacob SA. Simple practice guide for dose conversion between animals and humans. Journal of Basic and Clinical Pharmacy. 2016; 7(2)27-31

29. Bredt DS and Snyder SH. Isolation of nitric oxide synthase, a Calmodullin-requiring enzyme. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87;682-685

30. Li H and Fostermann U. Nitric oxide in the pathogenesis of vascular disease. Journal of Pathology. 2000; 190:244-252

31. Wang J, He Q, Yan X, Cai Y, Chen J. Effect of endogenous nitric oxide on sperm motility in vitro. Biological Research. 2014; 47(1)44 Doi:10.1186/0717-6287-47-44

32. Hou YC, Janczuk A and Wang PG. Current trends in the development of nitric oxide donors. Current Pharmaceutical Design. 1999; 5(6):417-441

33. Doshi SB, Khullar K, Sharma KS and Agarwal A. Role of reactive nitrogen species in male infertility. Reproductive Biology and Endocrinology. 2012; 10:109

34. Yadav S, Verma T, Pathak S and Nandi D. Understanding the roles of nitric oxide during sepsis and inflammatory disorders. In Morbidelli L and Bonavida B (eds) Therapeutic application of nitric oxide in Cancer and inflammatory disorders. Elsevier, 2012.

35. Aitken RJ and Curry BJ. Redox regulation of human sperm functions: from the physiological control of sperm capacitation to the etiology of infertility and DNA damage in the germ line. Antioxidants Redox Signal. 2011; 14(3)367-381