The Bioscan 8(1): 1005-1006, 2013 (Supplement on Toxicology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

HEAVY METAL INDUCED CHANGES OF RAT SERUM NITRITE AND NITRATE LEVELS

C. ASHOKA KUMAR, K. M. UDAY KUMAR, K. VIJAYA KUMARI AND M. RAJESWARA RAO*
Department of Zoology, S. V. University, Tirupati - 517 502, A.P.
e-mail:rajeswararao-m@hotmail.com

KEYWORDS
Heavy metals
Rat serum
Nitrite
Nitrate
Free radical damage

Received on :
13.04.2013

Accepted on :
12.07.2013

***Corresponding author**

ABSTRACT
Nitrite / Nitrate (NO^-_2/NO^-_3) are well known to be the end products of nitric oxide (NO) pathway, Keeping in view the toxic effects exerted by heavy metals, the impact of selected heavy metals on rat serum NO^-_3/NO^-_2 levels is reported. Selected heavy metals like Hg^{2+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Mn^{2+} and Al^{2+} were selected for the study and albino rats were treated with an IC_{50} dose of each metal. All the metals caused an elevation of rat serum NO^-_2/NO^-_3 levels of rat serum. It is reported that the heavy metals by way of enhancing rat serum NO^-_2/NO^-_3 levels may induce free radical type damage to various organs of rat.

INTRODUCTION

Alternations in the chemical composition of natural environment by industrial effluents like heavy metals usually induce changes in the behavioural, biochemical and pathological aspects of organisms (Boominathan and Ravendran, 2009). Nitric Oxide (NO), molecule of the mellenium (Shinde *et al.,* 2000) is well studied in recent years owing to its varied physiological functions in mammals. The very end products of NO are nitrite/nitrate (Guarner *et al.,* 1993). Earlier, from our laboratory, it is reported that certain of the heavy metals inhibit rat tissues cNOS and iNOS activities both *in vitro* and *in vivo* in experimental animals (Neelakantam, 2007). Present study is designed to investigate the *in vivo* effect of selected heavy metals on rat serum NO^-_2/NO^-_3 levels *in vivo*.

MATERIALS AND METHODS

Albino rats of the weight range 120 ± 5gr were selected for the present study. They were maintained at constant room temperature of 20 ± 5ºC and were fed *ad libitum* with commercial rat feed. They were divided into six groups of seven each.

Stock solutions of selected heavy metals like Hg^{2+}, $Pb^{2+}Cd^{2+}$, Cu^{2+}, Mn^{2+} and Al^{2+} were prepared in sterile water (1gr/2mL) and required IC_{50} concentration of each metal was prepared by diluting stock solutions with sterile water. The IC_{50} concentration selected for each metal is as shown below (in μmol).

Pb	Cd	Cu	Mn	Al	Hg
312.5	333.30	56.8	203.46	416.6	135.9

The above concentration of heavy metals were reported as IC_{50} Values that inhibit rat brain cNOS activity *in vitro* by Neelakantam (2007). Animals were gavaged with the above shown doses of each heavy metal. After 24h, the rat blood from the control and experimental ones was collected by cardiac puncture and were centrifuged at 2000xg for 15 minutes to collect the serum.

In the control and experimental samples the serum NO^-_3/NO^-_2 levels were determined following the procedure of Guarner *et al.* (1993). Statistical analysis was done using students 't' test.

RESULTS AND DISCUSSION

The results in Table 1 shows the levels of NO^-_3/NO^-_2 levels in IC_{50} dose heavy metal administered rat serum. In the control rat serum NO^-_2 levels were found to be more compared to NO^-_3 levels. All the heavy metals Screened enhanced the rat serum.

NO^-_3 and NO^-_2 levels and the changes were found to be statistically significant ($p < 0.001$) over the control ones. of all the metals Hg^{2+} was found to elevate the rat serum No^-_2/NO^-_3 levels and this was followed by $Pb^2 > Cd^{2+} > Cu^{2+} > Mn^{2+} > Al^{2+}$ (Table 1)

Once NO is produced, it diffuses into the mitochondrial matrix space and is further metabolized by reaction with O_2, ubiquinol and cytochrome oxidase to yield nitroxyl anion (NO) as intermediate and N_2O as stable final product (Arnaiz *et al.,* 1999). More accurate models of NO concentration profiles taking into account diffusional distribution of NO are available (Wood and Garthwaits, 1994). Imbalance of NO NO^-_2/NO^-_3 steady state concentrations, would contribute to

Table 1: Effect of methyl mercury on the Nitrate (NO^-_3) / Nitrite (NO^-_2) levels of rat serum (values expressed as mg of nitrate/mL of serum)

Name of the Metabolite	Control	Name of the heavy metal and their IC_{50} value					
		Hg^{2+}	Pb^{2+}	Cd^{2+}	Cu^{2+}	Mn^{2+}	Al^{2+}
NO^-_2							
AV	58.36	81.08*	77.51*	74.08*	70.11*	64.50*	60.36*
SD	±1.08	±3.46	±1.92	±0.82	±0.65	±0.92	±0.41
PC +		38.93	32.81	26.73	20.13	10.52	3.43
NO^-_3							
AV	40.75	71.22*	62.25*	58.51*	54.06*	48.84*	45.92*
SD	±0.54	±0.96	±1.22	±0.63	±0.88	±0.41	±0.62
PC +		74.77	52.76	43.58	32.66	19.85	12.68

Each value is the Mean ± SD of 5 Samples; AV = Average, SD = Standard Deviation, PC = Percent Change over the Control; * $p < 0.001$

altered H_2O_2 toxic effects. There are evidences in the literature in support of free radical formation from NO and there by causing cellular damage/ pathological conditions is well known (Poovala *et al.,* 1999; Ahmed *et al.,* 2000; Ali *et al.,* 2000). Like wise, in the current experimental results as shown in Table 1, elevated levels of rat serum NO^-_2/NO^-_3 levels in IC_{50} dose heavy metal treated rat serum may lead to either cellular damage or may cause some other pathological changes in the albino rats.

Earlier from over laboratory, we demonstrated that the pesticide treated rat serum exert higher levels of NO^-_3/NO^-_2 levels and the reasons were explained away as due to diffusion of NO from Cells/tissues of rat under pesticidal impact (Rao *et al.,* 1997) like reasons were also available from studies of the earlier authors (Wimc *et al.,* 1993). Similar reasons might be responsible for the elevated rat serum NO^-_2/NO^-_3 levels in heavy metal treated (rat serum) in the present study which in part becomes responsible for impairment of overall NO pathway in rats by heavy metals causing severe pathological defects in the albino rats.

REFERENCES

Ahmed, R. S., Vandana, S., Pasha, S. T., Benerjee, B. D. and Seth, V. 2000. Influence of dietary ginger (Zingiber officinates rocs) on oxidative stress induced by malathion in rats. *Food Chem. Toxicol.* **38(5)**: 443-450.

Ali, S., Wakar, G. and Pawa, S. 2000. Paraquat NO induces different pulmonary biochemical responds in wistar rats and swiss mice. *Chem. Boil, Interac.* **125(2)**: 79-91.

Arnaiz, S. L., Coronel, M. F. and Boveris, A. 1999. Nitric oxide, superoxide and hydrogen peroxide production in brain mitochondria after haloperidol treatment. Nitric oxide. *Biol. Chem.* **3(3):** 235-243.

Boominathan, M. and Ravendran, K. 2009. Impact of tannery effluent on biochemical responses in katla katla. *J. Ecotoxicol. Evron. Monior.* **19(2):** 113-117.

Guarner, C., Soriano, A., Tomas, O., Bulbena, M. T., Novella, I. *et al.* 1993. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxenia. *Hepatology.* **18:** 1139-1143.

Neelakantam, C. 2007. Impact of selected heavy metals on the nitric oxide pathway in rats, Ph. D., thesis, S.V. University, Tirupati. A. P.

Poovala, V. S., Hunag, H., Salahudeen, N. C. and Huans, H. 1999. Roles of reactive oxygen metabolites in organophosphate – bidrin – induced renal tubular cytotoxicity. *J. American, Soc. Nephrol.* **10(8)**: 1746-1752.

Rajeswara Rao, M., Kanji, V. K., and Sekhar, V. 1997. Pesticide induced changes of nitric oxide synthase in rat brain *in vitro*. *Drug. Chem. Toxicol.* **22:** 411-420.

Wink, D. A., Hanbaruer, J., Krishna, M. C., De Gralf, W. Gambson, J. and Mitchel, J. B. 1993. Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proc. Natl, Acad.Sci., U.S.A.* **90:** 9813-9817.

Shinde, V. A., Mehta, A. A. and Goyal, R. K. 2000. Nitric oxide: A molecule of the millennium. *Ind. J. Expl. Biol.* **38:** 201-210.