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Protection of Arsenic - Induced Thyroid Oxidative Stress by Melatonin

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Abstract
Arsenic-induced tissue damage is a major concern to the human population. An impaired antioxidant defense mechanism followed by oxidative stress is the major cause of arsenic-induced toxicity, which can lead to deleterious effects in the tissues. The present study was carried out to investigate the preventive role of melatonin, against arsenic-induced thyroid damage in mice. Administration of arsenic (in the form of arsenic trioxide, As_2O_3, at doses of 0.5 and 1.0 mg/kg body weight, po) for 30 days significantly decreased the intracellular antioxidant power, the activities of the antioxidant enzymes, as well as the level of total protein. In addition, arsenic intoxication enhanced lipid peroxidation and degeneration of the thyroid tissues. Concomitant administration of melatonin (at a dose of 10 mg/kg body weight, ip) with arsenic significantly restored all these parameters. In summary, the results suggest that the preventive role of melatonin against arsenic induced thyroid toxicity may be due to its intrinsic antioxidant property.

Keywords
Arsenic, Melatonin, Mice, Oxidative stress, Thyroid gland

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INTRODUCTION
Metal toxicity is a major medical concern. It has become evident that increasing human activities have modified the global cycle of heavy metals and metalloids, including the toxic non-essential elements like As, Hg, Cd and Pb. In the second half of the century pollution of air, water, soil and food, especially due to these 'big four' metals have become a threat to the plant and animal communities, including the human race. Among these metals, arsenic exhibits a complex metabolism and is possibly the most abundant pollutant and a potential human carcinogen. Exposure to high levels of arsenic in drinking water has been recognized for many decades in some regions of the world notably in China, India, Bangladesh and some countries in central and South America. Millions of people are at risk of cancer and various diseases because of chronic arsenic exposure. Adverse health effects that are associated with human exposure to arsenicals include hyperkeratosis, cardiovascular disease, developmental abnormalities, reproductive difficulties, neurological disorders, diabetes mellitus and cancers of the liver, kidney, lung and bladder (Tchounwou et al, 2012).

It is possible that impaired oxidant/antioxidant balance can be responsible for the toxic effect of Arsenic, hence, a therapeutic strategy to increase the antioxidant capacity of the cell is required to enhance the long-term effective treatment of arsenic poisoning. Several compounds have been tested against arsenic toxicity. In our quest for finding out an agent which could help in the amelioration of arsenic toxicity is essential to be worked out. Melatonin has several features that make it of clinical interest. It is readily absorbed when it is administered via any route, it crosses all morphological barriers, including the blood-brain barrier and placenta, with ease, it preserves mitochondrial function and it has low toxicity (Reiter et al., 2000; Acuna-Castroviejo et al., 2001). Therefore, our study has been done to test the causal relationship between arsenic generated oxidative stress and thyroid damage using mice as a model animal and further to examine whether melatonin interventions may be an effective strategy of detoxification that may help in preventing the damage induced by arsenic.

MATERIALS AND METHODS

Healthy, adult female albino mice (*Mus Musculus)* of Swiss strain weighing between 30-35g were procured under the Animal Maintenance and Registration No. 167/1999/ CPCSEA from the Ministry of Social Justice and Empowerment, Government of India. All the animals were acclimatized seven days prior to the commencement of the treatment. They were housed in air-conditioned animal house at a temperature of 26 ± 2°C and exposed to 10-12 hr. of daylight and a relative humidity of 30-70 %. Animals of different groups were caged separately and were maintained on standard chow (National Institute of Occupational Health (NIOH) containing wheat 70%, gram-20%, fish meat – 5% and yeast powder – 5%) and water was given *ad libitum.* Arsenic trioxide (As_2O_3) and Melatonin were purchased from Hi Media Laboratories Ltd., Mumbai, India. Stock solution of arsenic trioxide was prepared in double distilled water and orally given to mice with a hypodermic syringe. Both the doses for arsenic are derived from its LD_{50} value (39.4 mg/kg by. wt; Harrison *et al.,* 1958). Hence, low dose is $1/80^{th}$ of LD_{50}; while high dose is $1/40^{th}$ of LD_{50}. Animals were divided into following five groups: Group I served as untreated (control) animals, Group II were orally given 0.5 mg/kg by. wt As_2O_3 (Low dose – LD) while Group III were orally treated with 1 mg/kg by. wt As_2O_3 (High dose – HD) for 30 days. Group IV were given intraperitoneal injection of Melatonin alone (10 mg/kg by wt). Animals in Group V were administrated 1 mg As_2O_3 /kg (po) + 10 mg / kg by. wt Melatonin (i.p). Melatonin was administered at the same time each day. At the end of each treatment, the animals were weighed and then sacrificed using light ether anaesthesia. The thyroid gland of mice was dissected out carefully, blotted free of blood and weighed up to the nearest milligram and used for the study.

Lipid peroxidation (LPO) was determined by the method of Ohkawa *et al.,* (1979). The activities of catalase (E.C.1.11.1.6) and superoxide dismutase (SOD) (E.C.1.15.11) were analysed by the modified method of Luck, (1963), and spectrophotometric method of Kakkar *et al.,* (1984) respectively and glutathione (GSH) was estimated by the method of Grünert & Philips, (1951). The activity of glutathione peroxidase (GPx) (E.C.1.11.19) was assayed by the modified method of Paglia *&* Valentine*,* (1967). For all biochemical estimations a minimum of 8-10 replicates were done for each parameter. The data were expressed as mean ± SE (Standard error). For statistical analysis, the quantitative data of each parameter from the different groups were analyzed by Student's "t" test. The mean ± SE was calculated for each group and the corresponding level of significance was calculated.

RESULTS AND DISCUSSION

The LPO, GSH, GPx, CAT, SOD and protein levels of control and treated groups are given in Table 1. It was found that LPO level was higher ($P<0.001$) and GSH ($P<0.001$), GPx ($P<0.001$), CAT ($P<0.05$), SOD ($P<0.01$) as well as protein ($P<0.001$) levels were lower in arsenic treated mice compared with controls. Melatonin treatment significantly decreased the elevated LPO and significantly increased the lowered GSH, GPx, CAT, SOD and protein levels to control levels.

Arsenic trioxide induced alterations in antioxidant enzymes and lipid peroxidation in *Swiss* strain mice of this study appears to suggest that arsenic is able to cause oxidative stress in thyroid gland of these animals. Antioxidant enzymes such as CAT, SOD and GPx are the endogenous defenses; actively involved in scavenging of free radicals to maintain the steady state level and consequently integrity and functionality of cells. Superoxide dismutases are specific for catalytic removal of superoxides by converting them into H_2O_2 (Halliwell and Gutteridge, 2007). Hydrogen peroxide is catalytically dismutated by catalase into ground-state oxygen and water (Reiter et al., 2000). GSH is a predominant endogenous antioxidant and used as a cofactor to

remove hydrogen peroxide and lipoperoxides by the GPx family during which GSH is converted into oxidized form of glutathione (GSSG). Oxidized glutathione is converted back into GSH by another rate controlling enzyme the glutathione reductase thereby maintains the intracellular GSH levels. This optimum level of GSH is an utmost criterion in maintaining the structural integrity and physiology of cell membranes. GSH along with other endogenous antioxidants plays a central role in eliminating free radicals and other reactive species.

Arsenic-intoxication to mice in this experiment evidently induces decline in the activity of antioxidant enzymes (CAT, SOD, GPx) and level of GSH suggesting severe oxidative injuries to thyroid gland. Determination of activity level of these antioxidant enzymes is an appropriate indirect way to assess the pro-oxidant antioxidant status in tissues (Halliwell and Gutteridge, 2007; Priscilla and Prince, 2009). Studies by Zhang et al, 2017 also reported reduced activity of these enzymes by arsenic in the tissue in agreement with our data. The treatment of MLT is able to restore the level of antioxidant enzymes (Goc et al,2017) such as CAT, SOD and GPx, which are decreased in arsenic treated groups.

Table 1- Effect of Melatonin on biochemical and Antioxidative levels in Thyroid gland of arsenic exposed female mice. Group-I = Control; Group – II = Arsenic (LD); Group-III = Arsenic (HD) ; Group-IV = Melatonin ; Group –V = Arsenic + Melatonin (Values are Mean ± S.E.)

	Group-I	Group-II	Group-III	Group-IV	Group-V
Lipid peroxidation (MDA/mg tissue weight/60 min)	33.36 ± 0.73	58.89 ± 0.68^{c}	82.69 ± 0.84^{c}	33.13 ± 0.76^{d}	35.27 ±0.77^{d}
Glutathione (µg / 100 mg tissue weight)	31.58 ± 0.77	17.50 ± 0.66^{c}	10.81 ± 0.52^{c}	32.46 ± 0.75^{d}	30.08 ± 0.88^{d}
Glutathione peroxidase (nanomoles of NADPH oxidized / mg protein/min)	4.81 ± 0.30	3.10 ± 0.18^{c}	2.49 ± 0.31^{c}	4.41 ± 0.24^{d}	4.39 ± 0.31^{d}
Catalase (µ mol H_2O_2 consumed/mg protein/minute	8.12 ± 0.66	7.01 ± 0.55^{d}	5.87 ± 0.49^{a}	8.34 ± 0.51^{d}	7.39 ± 0.40^{d}
Superoxide dismutase (units/mg protein)	1.96 ± 0.25	1.24 ± 0.16^{a}	1.06 ± 0.16^{b}	2.04 ± 0.25^{d}	1.80 ± 0.24^{d}
Protein (mg/100mg)	15.14 ± 0.40	12.24 ± 0.28^{c}	11.84 ± 0.29^{c}	14.75 ± 0.44^{d}	15.61 ± 0.34^{d}

In each row, data having superscripts differ significantly from the corresponding control group.
P values : ^{a} < 0.05, ^{b} < 0.01, ^{c} < 0.001, ^{d} = Non Significant

Melatonin enhances cellular defence mechanism-it specifically augments the activity of antioxidant enzymes, such as glutathione reductase and glutathione peroxidase (Okatani et al.,2001). These enzymes convert oxidized glutathione to GSH. The protective effects of MLT in maintaining the GSH level towards control have increased the steady state of GSH and its rate of synthesis that confers enhanced protection against oxidative stress. Zhang et al.,2017 also reported that co-treatment with melatonin normalized the level of non-enzymatic antioxidants such as glutathione suppressed by arsenic. Lipid peroxidation is regarded as one of the basic mechanisms of cellular damage caused by free radicals. In the present study, groups treated with arsenic alone are more vulnerable to oxidative injuries and thereby increase in the lipid peroxidation, whereas the group received the co-administration of melatonin exhibited significant protection. In support to our data, studies by Bharti and Srivastava, 2014; Zasada and Karbwonik, 2015 also reported altered LPO after melatonin treatment. Altered protein levels in thyroid also induced changes leading to biochemical defects, structural disorders and altered physiological functions. A number of sulfhydryl containing proteins and enzyme systems have been found to be altered by exposure to arsenic (Robert & Judd, 1986; Roy &

Saha, 2002) Thus a significant reduction in protein level in our study indicates toxicity status of the tissue by arsenic (Jhala et al, 2004) which binds to -SH groups leading to protein synthesis inhibition. The present findings hence revealed that arsenic caused formation of free radicals in the thyroid gland by reducing the antioxidant indices. However, melatonin supplementation to arsenic fed mice exhibited no effects in thyroid antioxidant system indicating its antioxidative property. This antioxidant thus ameliorates metal oxidative thyroid damage and might have a therapeutic use in heavy metal induced oxidative stress in human.

CONCLUSION

In vivo study revealed that exposure of arsenic caused oxidative stress in thyroid gland of *Swiss* mice but supplementation of MLT restored altered biochemical variables by its antioxidative potential.

REFRENCES

1. Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. In *Molecular, clinical and environmental toxicology* (pp. 133-164). Springer, Basel.
2. Reiter, R. J., Tan, D. X., Osuna, C., & Gitto, E. (2000). Actions of melatonin in the reduction of oxidative stress. *Journal of biomedical science*, *7*(6), 444-458.
3. Acuña-Castroviejo, D., Martín, M., Macías, M., Escames, G., León, J., Khaldy, H., & Reiter, R. J. (2001). Melatonin, mitochondria, and cellular bioenergetics. *Journal of pineal research*, *30*(2), 65-74.
4. Harrison J W E, Packman E W & Abbott D D, Acute oral toxicity and chemical and physical properties of arsenic trioxides *Arch Ind Health*, 17 (1958) 118.
5. Ohkawa H, Ohishi N & Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Anal Biochem*, 95 (1979) 351.
6. Luck H, Catalase, in *Methods of Enzyme Analysis*, edited by HU Bergmeyer, (Academic Press, New York) 1971,885
7. Kakkar P, Das B & Vishwanathan P N, A modified spectrophotometric assay of superoxide dismutase, *Indian J Biochem Biophys*, 21 (1984) 130.
8. Grunert R R & Philips P H, A modification of nitroprusside method of analysis for glutathione, *Arch Biochem Biophys*, 30 (1951) 217.
9. Pagila D E & Valentine W N, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxides, *J Lab Clin Med*, 70 (1967) 158.
10. Halliwell, B., & Gutteridge, J. M. C. (2007). Antioxidant defenses endogenous and diet derived. *Free radicals in biology and medicine*, *111*.
11. Priscilla, D. H., & Prince, P. S. M. (2009). Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. *Chemico-biological interactions*, *179*(2-3), 118-124.
12. Zhang, Y., Wei, Z., Liu, W., Wang, J., He, X., Huang, H., ... & Yang, Z. (2017). Melatonin protects against arsenic trioxide-induced liver injury by the upregulation of Nrf2 expression through the activation of PI3K/AKT pathway. *Oncotarget*, *8*(3), 3773.
13. Okatani, Y., Wakatsuki, A., Shinohara, K., Kaneda, C., & Fukaya, T. (2001). Melatonin stimulates glutathione peroxidase activity in human chorion. *Journal of pineal research*, *30*(4), 199-205.
14. Bharti, V. K., Srivastava, R. S., Kumar, H., Bag, S., Majumdar, A. C., Singh, G., ... & Brown, G. M. (2014). Effects of melatonin and epiphyseal proteins on fluoride-induced adverse changes in antioxidant status of heart, liver, and kidney of rats. *Advances in pharmacological sciences*, *2014*.
15. Zasada, K., & Karbownik-Lewinska, M. (2015). Comparison of potential protective effects of melatonin and propylthiouracil against lipid peroxidation caused by nitrobenzene in the thyroid gland. *Toxicology and industrial health*, *31*(12), 1195-1201.
16. Goc, Z., Szaroma, W., Kapusta, E., & Dziubek, K. (2017). Protective effects of melatonin on the activity of SOD, CAT, GSH-Px and GSH content in organs of mice after administration of SNP. *Chin. J. Physiol*, *60*(1), 10p.
17. Robert EM & Judd O N. (1986) Water and soil pollutants, in *Toxicology-The basic science of poison*, edited by C D Klassen, M D Ambur & J Doull, (Macmillan Publishing Company), 825.
18. Roy, P., & Saha, A. (2002). Metabolism and toxicity of arsenic: A human carcinogen. *Current science*, 38-45.
19. Jhala, D. D., Nair, S. B., & Chinoy, N. J. (2004). Reversible toxicity of fluoride and arsenic in ovary of mice. *Fluoride*, *37*(2), 71-79.