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MELATONIN MODULATES MERCURY INDUCED OXIDATIVE STRESS IN RAT LIVER

Tapan A. Patel[1]* and Mandava V. Rao[2]

[1]Department of Biology, Indian Institute of Teacher Education, Ramkrishna Paramhans Vidya Sankul, 'KH' Road, Nr. Mahatma Mandir, Sector-15, Gandhinagar, Gujarat, India.

[2]Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380009, Gujarat, India.

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***Correspondence for Author**
Dr. Tapan A. Patel
Department of Biology, Indian Institute of Teacher Education, Ramkrishna Paramhans Vidya Sankul, 'KH' Road, Nr. Mahatma Mandir, Sector-15, Gandhinagar, Gujarat, India.

ABSTRACT

Objective: The present work was aimed to assess the beneficial effect of melatonin against mercury induced oxidative stress in rat liver. **Methods:** Rats were divided into five groups and treated for 60 days. Group 1 served as control. Group 2 & 3 animals were treated with mercuric chloride (2 & 4mg/kg BW) orally. Group 4 animals were treated with melatonin (5mg/kg BW) intraperitoneally and Group 5 animals received melatonin with $HgCl_2$. Various antioxidant indices assayed were superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST), thiobarbituric acid-reactive substances (TBARS), total -SH, glutathione (GSH), protein carbonyl (PC), hydrogen peroxide (H_2O_2) and total ascorbic acid (TAA). **Results:** Treatment of $HgCl_2$ caused significant ($P<0.01$, $P<0.001$) decrease in activities of SOD, CAT, GPx and GR as well as levels of total -SH, GSH, TAA and total proteins reduced significantly ($P<0.001$). Whereas, the levels of TBARS, PC and H_2O_2 showed significant elevation. Administration of melatonin with mercury mitigated significantly the decline in activities of SOD, CAT, GPx and GR as well as restored the levels of total -SH, GSH, TAA, total proteins, TBARS, PC and H_2O_2. **Conclusion:** The results concluded that melatonin supplementation modulated mercuric chloride induced hepatotoxicity.

KEYWORDS: Melatonin, mercuric chloride, oxidative stress, hepatotoxicity, rat.

1. INTRODUCTION

The liver is a vital organ of the body involved in detoxification, protein synthesis and the process of digestion. While performing its function, especially as regards exogenous agents, it may be damaged. Common causes of liver damage include xenobiotics, alcohol consumption, malnutrition, infection, anemia and medications.[1]

Various heavy metals are widely found in our environment. Living organisms are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Mercury is released into the environment through various processes, which may be natural and/or anthropogenic.[2] Mercury compounds can inactivate number of enzymes by blocking the functional sites i.e. -SH groups, which are part of the catalytic or binding domains. All forms (elemental, inorganic and organic) of mercury cause toxic effects in a number of tissues, organs and chromosomes depending on the chemical form of mercury as well as the level, duration and the route of exposure.[3,4] The high mobility of mercury in the body is attributed due to the formation of water-soluble mercury complexes that are mainly attached to the sulfur atom of thiol groups such as glutathione.[5] Administration of mercury to male wistar rats, showed hepatotoxicity by alterations in the antioxidant system as well as in biochemical indices.[6,7] Nowadays, much attention has been focused on the protective effect of antioxidants in biological systems against different toxic heavy metals.

Melatonin (N-acetyl-5-methoxy tryptamine) is a hormone secreted by the pineal gland.[8] Melatonin belongs to an amino group, more specifically to the indolamine group, since it is derived from the aminoacid tryptophan.[9] It is a powerful free radical scavenger and also capable of eradicating H_2O_2, ·OH, peroxinitrite anion (ONOO-), singlet oxygen and peroxyl radical (LOO·). There are various mechanisms embodying melatonin efficiency to overcome oxidative stress are: 1) direct free radical scavenging actions,[10,11] 2) enhancing the activities of antioxidative enzymes,[12,13,14] 3) stimulating actions on the synthesis of non-enzymatic intracellular antioxidant i.e. glutathione (GSH)[15] and 4) reducing electron leakage from the mitochondrial electron transport system.[16] Melatonin detoxifies numerous reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), hydroxyl radical (OH-), peroxyl radicals (ROO-), singlet oxygen (O2-) and also reactive nitrogen species (RNS) such as nitric oxide radical (NO-) and peroxinitrite (ONOO-). It also neutralizes hypochlorous acid.[17,18] Melatonin also prevents the damage caused by oxidative stress at the molecular, cellular, tissue, organ and organ system levels.[11]

However, the role of melatonin on mercury exerted hepatotoxicity has attained less attention. Hence, this study was conducted to investigate the protective effect of melatonin against oxidative stress induced by mercury in the rat liver.

2. MATERIALS AND METHODS

2.1. Chemicals

Melatonin, mercuric chloride and all the other chemicals used in the experiment, were of the highest analytical grade and purchased from HiMedia (Mumbai).

2.2. Animals and treatments

Male Wistar Albino rats (*Rattus norvegicus*), weighing 200-250gms were obtained from Cadila Pharma, under the Animal maintenance and Registration No.167/1999/CPCSEA from the ministry of Social Justice and empowerment, Govt. of India. The rats were housed in the plastic cage with good ventilation and were fed on the standard commercial laboratory chow with distilled water *ad libitum*. Light dark conditions as well as the temperature was maintained (12h: 12h and 26±2^{o}C respectively) throughout the seasons. After one week of acclimatization, rats were divided into five equal groups of 10 rats each. **Group I** served as control and animals were provided with distilled water. **Group II** animals were treated with the low dose (2mg/kg body weight) of $HgCl_2$ orally by gastric gavage. **Group III** animals were also treated with the high dose (4mg/kg body weight) of mercuric chloride (MC) orally. **Group IV** animals were treated with melatonin (MLT-5mg/kg body weight) intraperitoneally and **Group V** animals received melatonin half an hour before the treatment of high dose of $HgCl_2$. Because of fast metabolism of melatonin, it was administered 25-30 mins before the $HgCl_2$ treatment.[19] Animals were treated regularly and daily for 60 days.

2.3. Collection and processing of tissues

On the 61st day, the animals were weighed and necropsy was performed. The liver samples were removed carefully and washed with ice-cold normal saline solution and placed on ice. The tissues were homogenized using a tissue homogenizer in the respective buffers as per the protocol and the various assays were performed within 24 h of the animal dissection.

2.4. Enzymatic-antioxidant indices

The tissue homogenate was used for spectrophotometric estimation of various antioxidant enzymatic indices like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase

(GPx), glutathione reductase (GR), glutathione-s-transferase (GST) by different methods.[20,21,22,23,24]

2.5. Nonenzymatic-antioxidant indices

The estimation of levels of total thiol (-SH), glutathione (GSH), Total ascorbic acid (TAA), Thiobarbituric acid-reactive substances (TBARS), protein carbonyl (PC), hydrogen peroxide (H_2O_2) was carried out by various methods.[25,26,27,28,29,30] Total proteins were determined by the method of Lowry et al., 1951.[31]

2.6. Statistical analysis

All data were analyzed by SPSS software (ver. 16). The results were articulated as mean ± SEM. Means of individual groups were compared by student's t-test. The data were analyzed using one way analysis of variance (ANOVA) followed by *Post hoc* testing for intergroup comparisons. Values of $p<0.05$ were considered significant. The percent amelioration was also calculated by using the following formula.[32]

% amelioration = (pro oxidant group – respective antioxidant group) / (pro oxidant group – Control) × 100.

3. RESULTS

3.1. Enzymatic antioxidant findings

As shown in Table 1, activities of various antioxidant enzymes such as SOD, CAT, GPx, and GR were significantly ($P<0.01$, $P<0.001$) decreased compared to control after mercury treatment. The treatment, contrarily, significantly ($P<0.001$) enhanced the activity of GST.

On the other side, administration of melatonin alone and in combination with the high dose of mercury did not reveal any significant difference in the activity of these enzymes when compared to control values. Melatonin treatment in combination with high dose of mercury showed 60 to 95 % amelioration among these indices.

3.2. Nonenzymatic antioxidant findings

Table 2 showed that after mercury treatment levels of total thiol (-SH), GSH and TAA reduced significantly ($P<0.001$). Contrarily, the levels of TBARS, PC and H_2O_2 showed significant elevation compared to control after mercury treatment.

However, melatonin treatment alone did not reveal significant alteration. Combination of melatonin and mercury high dose showed amelioration and did not reveal significant changes

compared to control values. As shown in Table 2, combination of melatonin and mercury high dose revealed 55 to 95% amelioration among nonenzymatic indices.

The level of total proteins showed marked, significant ($P<0.001$) decline (Table 2) after mercury treatment, whereas the treatment of melatonin and high dose of mercury in combination revealed 74.80% amelioration.

Table 1: Oxidative stress parameters in liver of control and treated animals.

Parameters	Group I	Group IV	Group II	Group III	Group V	
	Control	Melatonin	L.D.	H.D.	Melatonin + H.D.	% Amelioration
			$HgCl_2$			
		Treated for 60 Days				
Superoxide dismutase [a]	8.15 ± 0.18	7.79 ± 0.16^{NS}	6.06 ± 0.18^{++}	4.59 ± 0.19^{++}	7.43 ± 0.24^{NS}	79.77
Catalase [b]	26.47 ± 2.65	27.35 ± 2.20^{NS}	19.93 ± 2.04 ^{NS}	14.70 ± 1.14**	21.99 ± 1.41^{NS}	61.93
Glutathione peroxidase [c]	26.16 ± 1.08	25.49 ± 1.43^{NS}	16.86 ± 2.72*	9.80 ± 1.77^{++}	25.42 ± 1.65^{NS}	95.47
Glutathione reductase [d]	35.34 ± 0.64	32.22 ± 0.90^{NS}	28.28 ± 0.23^{++}	16.48 ± 0.46^{++}	30.23 ± 1.35*	72.90
Glutathione S transferase [e]	0.0214 ± 0.00008	0.0202 ± 0.0006^{NS}	0.0330 ± 0.00120^{++}	0.0408 ± 0.0005^{++}	0.0265 ± 0.0015*	73.71

NS = Non Significant, * = $p < 0.05$, ** = $p < 0.01$, + = $p < 0.005$, ++ = $p < 0.001$ (Groups II to V compared with control Group I)
Values are Mean ± S.E.
a = units/mg protein, b = µmoles of H_2O_2 consumed/min/mg protein, c = µmoles of GSH consumed/min/mg protein, d = nmoles of NADPH oxidized/min/mg protein, e = units/mg protein.

Table 2: Non-enzymatic antioxidant parameters in liver of control and treated animals.

Parameters	Group I	Group IV	Group II	Group III	Group V	
	Control	Melatonin	L.D.	H.D.	Melatonin + H.D.	% Amelioration
			$HgCl_2$			
		Treated for 60 Days				
Total -SH [a]	2.98 ± 0.11	2.84 ± 0.29^{NS}	$1.88 \pm 0.16^{+}$	$1.27 \pm 0.21^{++}$	2.40 ± 0.17^{NS}	66.08
Glutathione [b]	88.0 ± 5.83	82.0 ± 7.18^{NS}	$44.8 \pm 2.05^{++}$	$29.6 \pm 4.16^{++}$	62.6 ± 5.24^{NS}	56.50
Total Ascorbic Acid [c] **(TAA)**	3.01 ± 0.18	3.13 ± 0.18^{NS}	$1.42 \pm 0.036^{++}$	$0.57 \pm 0.01^{++}$	2.80 ± 0.11^{NS}	91.39
TBARS [d]	31.45 ± 1.65	30.50 ± 4.68^{NS}	$43.39 \pm 1.9^{**}$	$53.50 \pm 2.37^{++}$	35.16 ± 2.42^{NS}	83.17
Protein Carbonyl [e]	7.20 ± 0.31	7.01 ± 0.33^{NS}	$9.43 \pm 0.35^{**}$	$11.78 \pm 0.21^{++}$	7.49 ± 0.19^{NS}	93.66
Hydrogen peroxide [f]	36.30 ± 6.90	34.10 ± 3.28^{NS}	$72.20 \pm 3.33^{**}$	$83.80 \pm 3.09^{+}$	48.60 ± 0.81^{NS}	74.10
Total proteins [g]	15.72 ± 0.27	15.37 ± 0.64^{NS}	$9.43 \pm 0.79^{++}$	$6.87 \pm 0.76^{++}$	13.49 ± 0.93^{NS}	74.80

NS = Non Significant, * = $p < 0.05$, ** = $p < 0.01$, + = $p < 0.005$, ++ = $p < 0.001$ (Groups II to V compared with control Group I)
Values are Mean ± S.E.
a = mg/ 100 mg tissue weight, b = µmoles/100mg Tissue weight, c= mg/gm Tissue weight, e = n moles /mg protein, g = mg/100mg tissue weight, d = n moles of MDA formed/100mg tissue weight, f = nmoles H_2O_2/h/gm tissue weight, TBARS = Thiobarbituric acid reactive substances.

4. DISCUSSION

$HgCl_2$ is one of the deleterious forms of mercury since it can easily interact with proteins,[33] leading to morphological and physiological alterations in many organ systems, such as central nervous system, pulmonary system, urinary system, endocrine system and reproductive system.[34,35,36] However, according to Sundal et al., 2005[37] production of reactive oxygen species (ROS) is an essential process of metabolism. Oxidative stress is a condition resulted due to overwhelming ROS levels in the body. Unchecked excessive ROS can lead to the destruction of cellular compounds, including lipids, proteins and DNA resulting in changes in cell permeability, ultimately leading to cell death.[38]

In the present study, chronic administration of mercury to rats caused toxic effects in the liver via generating the ROS and affecting its functions. One of the factors that induced free radicals is lipid peroxidation, which breaks unsaturated fatty acids of the cell membrane. Thus, the membrane permeability and function gets altered due to enhanced TBARS levels as a result of toxicity. In our study, these levels were increased in the liver tissue due to mercury poisoning. An increase in the levels of TBARS due to mercury intoxication was also observed in different rat tissues[39,40] in corroboration of our data.

SOD, GR and GPx are important antioxidant enzymes and form the first line of defense against oxidative stress. Inorganic mercury treatment increased production of free radicals by impairing the efficiency of oxidative phosphorylation and the electron transport system.[41] The enzyme SOD catalyses the superoxide dismutation to O_2 and H_2O_2. GPx and CAT convert H_2O_2 further to H_2O.[42] The decreased activity of SOD and GPx in the liver and other organs leading to the accumulation of superoxide anion radical, which might be responsible for increased lipid peroxidation following the $HgCl_2$ treatment. The amount of glutathione in the liver diminished after mercury treatment in light of our data.[2,39] The reduction in the activity of GR and GPx also supported changes in glutathione metabolism leading to oxidative stress in the liver. The role of glutathione and its related enzymes like GR and GPx is well known in stress condition and are responsible for the defense mechanism.[43] Thus, mercury brought about an imbalance in intracellular redox status leading to oxidative stress. Agarwal & Behari (2007)[2] reported that in the liver after mercury treatment, the activity of SOD, GPx and CAT altered significantly in support of our data. $HgCl_2$ treatment brought a decrease in protein levels, in support of our data, due to its oxidation via ROS affecting thiol (-SH) groups containing molecules such as glutathione, cysteine as well as -SH groups of

proteins. Hoffman & Heinz (1998)[44] reported reduction in total thiol groups and protein content in liver after mercury treatment which supports our data. In the present study, the decreased level of hepatic total thiol groups in $HgCl_2$ treated rats may be due to excessive utilization in detoxification of mercury.

Production of large amounts of ROS damages the protective system, leading to various structural alterations (viz. oxidation) in proteins.[45] The oxidation of proteins, to form carbonyls, occurs via the hydroxyl radical, since neither H_2O_2 nor superoxide is reactive enough to provoke oxidation. Hence, carbonyl content is a sensitive indicator of oxidative damage to proteins.[46] Mercury treatment significantly increased protein carbonyl content in various tissues in corroboration of our data.[47,48]

Vitamin C (ascorbic acid) scavenges ROS by quick electron transfer and thus inhibits lipid peroxidation as well as reduces the oxidized tocopheroxyl radicals. Thus, it protects membrane lipids from damage.[49] It is well ascertained that the non-enzymatic antioxidants such as vitamin C concomitantly decreased along with thiol groups in heavy metal toxicity in support of our data.[50,40,48]

In the present study administration of melatonin declined the oxidative stress in mercury treated rats. Many studies have reported protective actions of melatonin against oxidative stress due to its high efficacy as a free radical scavenger and an indirect antioxidant in support of our data.[51] Increased levels of LPO and TBARS due to oxidative stress were also declined after melatonin administration. Saad et al. (2013)[52] reported that melatonin significantly reduced hepatotoxicity caused due to oxidative stress. It can also improve the reduced activity of various antioxidant enzymes viz., SOD, CAT, GPx, GR etc. in different tissues.[35,53] Levels of glutathione and total thiol groups were also improved after melatonin supplementation in present study. There are several reports documented that melatonin can restore cellular GSH level of tissues in various models of oxidative stress in corroboration of our data, possibly due to its stimulatory effect on GSH synthesis.[15] Melatonin supplementation reduced LPO and increased level of reduced glutathione, showing a significant improvement of the oxidative stress.[54,55] It protects the level of vitamin C, reduced after metal intoxication in the rat.[48,56] Melatonin seems to be potent in either scavenging the toxic free radicals and other reactive intermediates[57] or chelating the redox-active transition/non-transition heavy metal divalent cations, thus protecting the essential

cellular proteins from oxidation.[58] In corroboration of our data, melatonin attenuated protein carbonyl formation after heavy metal intoxication in rats.[48,56]

CONCLUSION

In conclusion, the results of the present study demonstrate that melatonin showed a significant protective action against mercury induced hepatotoxicity in rats via increasing the endogenous antioxidant defense systems (enzymatic and non-enzymatic) as well as inhibiting the lipid peroxidation and protein carbonyl formation. Accordingly, it may be suggested that melatonin can serve as a potential therapeutic candidate for the liver injury associated with heavy metal induced oxidative stress.

Conflict of interest statement

We declare that we have no conflict of interest.

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