

## Mercury Chloride-Induced Glucose Intolerance in Rats: Role of Oxidative Stress

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**Abstract:** Mercury is recognized as a hazardous environmental and industrial pollutant; however it is commercially important in many industries/applications. This has therefore led to an increase in occupational and environmental exposures in many parts of the world. The present study evaluated the impact of mercury chloride on glucose homeostasis and the possible involvement of oxidative stress. In addition, the potential protective effects of Alpha Lipoic Acid (ALA), a naturally occurring and unique antioxidant on mercury chloride toxicity were investigated. Thirty rats were randomly divided into five equal groups of six animals: Group 1 (control) received 0.5 mL distilled water; Group 2 (ALA) received 100 ug/kg of ALA; Group 3 (LDM) and Group 4 (HDM) received 250 and 500 ug/kg body weight of HgCl<sub>2</sub>, respectively; and Group 5 (HDM-ALA) received 500 ug/kg body weight of HgCl<sub>2</sub> simultaneously with 100 ug/kg of ALA. Both HgCl<sub>2</sub> and ALA were administered orally for 14 consecutive days. The fasting blood glucose was measured and all groups were subjected to Oral Glucose Tolerance Test (OGTT). In addition, the activities of reduced glutathione, superoxide dismutase, catalase as well as the level of lipid peroxidation were estimated. The results showed significant adverse changes in glucose tolerance and oxidative indices in rats exposed to mercury chloride. However, treatment with ALA attenuated all mercury triggered changes. This study clearly demonstrates the induction of glucose intolerance by mercury chloride and suggests the involvement of oxidative stress as an important regulator of glucose homeostasis during mercury chloride exposure.

**Key words:** Antioxidants and lipid peroxidation, diabetes mellitus, glucose tolerance, mercury chloride, oxidative stress

### INTRODUCTION

Mercury is a highly toxic metal that exerts its adverse effect on health of humans and animals through air, soil water and food. Although, mercury has been recognized as a hazardous environmental and industrial pollutant (WHO, 1991), it is however commercially important in many industries, and their occupational and environmental exposures continue to increasingly occur in many parts of the world (Järup, 2003). The most frequent chemical forms to which humans and animals are exposed include elemental mercury vapour, mercuric salts as mercuric chloride (HgCl<sub>2</sub>) and organic mercury compounds such as methyl mercury (CH<sub>3</sub>Hg) (Drasch *et al.*, 2001). Its application is found in agriculture as fungicide, in medicine as topical antiseptic, disinfectant, parasiticidal as well as amalgam fillings in dentistry (ATSDR, 1999; Jagadeesan, 2004). In addition, it is also found in scientific instruments, electrical equipment, disk batteries, caustic soda and the atmosphere (Aschner and Walker, 2002). These various sources account for the accidental, occupational and environmental exposures to mercury. Meanwhile, one of the harmful effects of mercury action during its accumulation in the body is the

excessive release of Reactive Oxygen Species (ROS) and increased lipid peroxidation in the cells (Durak *et al.*, 2010; Sharma *et al.*, 2007). The formation of (ROS) in cells leads to the formation of radicals in metabolic processes which causes damage to many molecules in cells, including membrane lipids, proteins and nucleic acids (Ilker *et al.*, 2004), which can lead to the development of many pathological process (Gutteridge, 1993). These harmful effects are controlled by antioxidant defense system in cells which include enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose -6- phosphate dehydrogenase (Erat *et al.*, 2007; Faix *et al.*, 2003).

Oxidative stress which usually results from excessive production of ROS and/or diminished activity of antioxidants (Halliwell and Gutteridge, 1999) have been implicated as a major contributor to the aetiology of severe pathologies, including diabetes (Perez-Matute *et al.*, 2009). Moreover, increasing evidence shows that excess ROS acts as negative regulators of insulin signaling leading to insulin resistance, a known metabolic abnormality associated with diabetes (Valko *et al.*, 2007; Bashan *et al.*, 2009). Previous studies have also shown that group IIb metals (cadmium, mercury and zinc)

stimulate glucose transport in adipocytes (Barnes *et al.*, 2003; Tang and Shay, 2001). Barnes and Kircher (2005) reported that pre-treatment with HgCl<sub>2</sub> decreased insulin-mediated glucose transport 1.3-fold which is a characteristic of insulin resistance. Nevertheless, it remains to be determined if exposure to mercury chloride will cause any glucose pathologies in systemic environment and the possible mechanism of action if it does. The present study was therefore undertaken to evaluate the impact of mercury chloride on glucose homeostasis in Sprague Dawley rats and the possible involvement of oxidative stress. In addition, the potential protective effects of Alpha Lipoic Acid (ALA), a naturally occurring and unique antioxidant on mercury chloride toxicity were investigated. For this purpose, the lipid peroxidation level; superoxide dismutase, catalase and reduced glutathione activities; as well as the glucose level and tolerance were determined.

## MATERIALS AND METHODS

**Drugs and chemical reagents:** ALA, HgCl<sub>2</sub>, and olive oil were obtained from Sigma (USA). All other chemicals and test kits used were of analytical grade.

**Animals:** Male Sprague-Dawley rats weighing 140-170g were obtained from the Laboratory Animal House of the College of Medicine of the University of Lagos. Animals were allowed to acclimatize for seven days before the commencement of the experiment. They were fed with standard pellet diet and water *ad libitum* at 20-25°C under a 12 h light/dark cycle. All animal handling and experiment protocols complied with the international guidelines for laboratory animals.

**Experimental groups:** Thirty (30) animals were randomly divided into five equal groups of six animals: Group 1 (control) received 0.5 mL distilled water; Group 2 (ALA) received 100 ug/kg of ALA; Group 3 (LDM) and Group 4 (HDM) received 250 and 500 ug/kg body weight of HgCl<sub>2</sub>, respectively; and Group 5 (HDM-ALA) received 500 ug/kg body weight of HgCl<sub>2</sub> simultaneously with 100 ug/kg of ALA; both HgCl<sub>2</sub> and ALA were administered orally for 14 consecutive days.

**Oral glucose tolerance test:** All groups were subjected to oral glucose tolerance test (OGTT). The rats were fasted overnight for sixteen-hour (16-h) and subsequently challenged with a glucose load of 2 ug/kg body weight. Blood glucose levels were determined at 0 h (pre-glucose treatment) and at 30, 60, 90, 120 and 180 min (post-glucose treatment). The glucose levels were measured using a complete blood glucose monitoring system (One-Touch Ultra Easy Glucose Meter, Lifescan, U.K.).

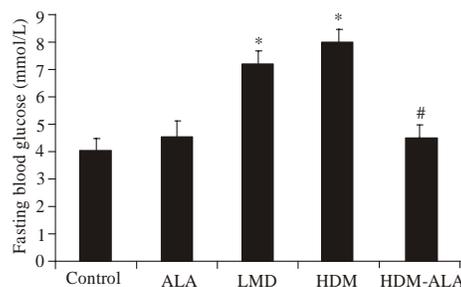


Fig. 1: Effect of ALA on fasting blood glucose in control and experimental rats. Values are expressed as mean±SEM (n = 6). \*: p<0.05 when compared with control group; #: p<0.05 when compared with mercury treated groups.

**MDA level:** As a marker of lipid peroxidation, the level of malondialdehyde (MDA) in the liver homogenate was measured by the method of Uchiyama and Mihara (1978) as Thiobarbituric Acid Reactive Substances (TBARS). The development of a pink complex with absorption maximum at 535 nm is taken as an index of lipid peroxidation.

**SOD, CAT and GSH activities:** The activity of the superoxide dismutase (SOD) enzyme in the liver homogenate was determined according to the method described by Sun and Zigmam (1978). The reaction was carried out in 0.05M sodium carbonate buffer pH 10.3 and was initiated by the addition of epinephrine in 0.005N HCl. Catalase (CAT) activity was determined by measuring the exponential disappearance of H<sub>2</sub>O<sub>2</sub> at 240nm and expressed in units/mg of protein as described by Aebi (1984). The reduced glutathione (GSH) content of the liver homogenate was determined using the method described by Van Dooran *et al.* (1978). The GSH determination method is based on the reaction of Ellman's reagent 5, 5' dithiobis-2-nitrobenzoic acid (DNTB) with the thiol group of GSH at pH 8.0 to produce 5-thiol-2-nitrobenzoate which is yellow at 412 nm. Absorbance was recorded using Agilent UV-Visible Spectrophotometer in all measurement.

**Statistical analysis:** Data were presented as mean and Standard Error of Mean (SEM). When one-way ANOVA showed significant differences among groups, Tukey's *post hoc* test was used to determine the specific pairs of groups that were statistically different. A level of p<0.05 was considered statistically significant. Analysis was performed with the GraphPad InStat Version 3.05 (GraphPad Software, San Diego California, USA).

## RESULTS

**Fasting blood glucose:** Figure 1 shows significant increases in the fasting blood glucose level of both LDM and HDM rats when compared with the control. However,

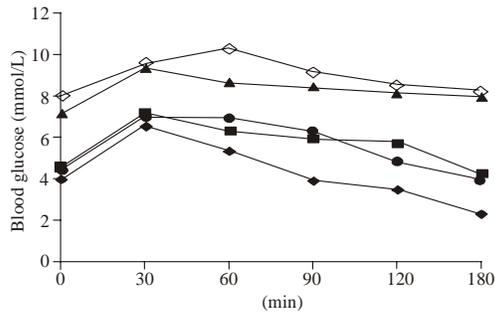


Fig. 2: Effect of ALA on OGTT in control and experimental rats. Values are expressed as mean (n = 6)

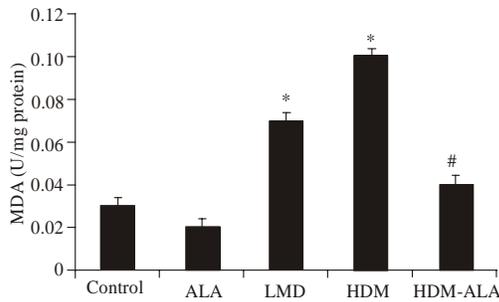


Fig. 3: Effect of ALA on MDA level in control and experimental rats. Values are expressed as mean±SEM (n = 6). \*: p<0.05 when compared with control group; #: p<0.05 when compared with mercury treated groups

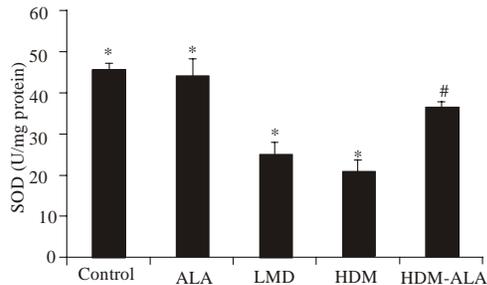


Fig. 4: Effect of ALA on SOD activity in control and experimental rats. Values are expressed as mean±SEM (n = 6). \*: p<0.05 when compared with control group; #: p<0.05 when compared with mercury treated groups

HDM-ALA rats showed a significant decrease in blood glucose level when compared with both LDM and HDM rats. Similarly, ALA rats also showed a significant decrease in the fasting blood glucose level compared with both LDM and HDM. There was no significant difference in the blood glucose level of LDM and HDM when compared with the control rats.

**Oral glucose tolerance test:** The glucose tolerance ability of the LDM and HDM rats was significantly lower

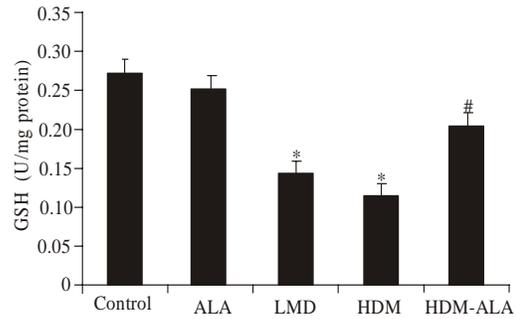


Fig. 5: Effect of ALA on GSH activity in control and experimental rats. Values are expressed as mean±SEM (n = 6). \*: p<0.05 when compared with control group; #: p<0.05 when compared with mercury treated groups

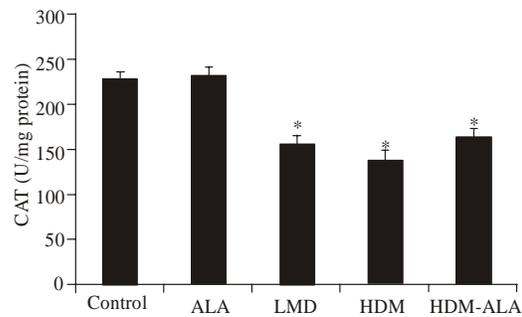


Fig. 6: Effect of ALA on CAT activity in control and experimental rats. Values are expressed as mean±SEM (n = 6). \*: p<0.05 when compared with control group

than that of the control rats in the 30-180 min period of OGTT. Improved glucose tolerance was however observed in the mercury-exposed rat treated with ALA during the 30-180 min after glucose load compared with the corresponding HDM rats without ALA. The results of OGTT as presented in Fig. 2 showed that there was a 1.7-fold increase in the blood glucose level after 30 min of oral glucose load in control rats, and a fall in the blood glucose level was observed at 60 min post glucose load. At 120 min, the blood glucose level was back to normal, while further decline in the glucose level were observed at 120-180 min period. The data from the LDM and HDM rats showed a 0.5-fold rise in the blood glucose level after 30 min of oral glucose load. At 60 min, there was a sustained increase in the blood glucose level in the HDM rats while there was a decrease in the LDM rats. At 90-120 min period, there was a fall in the blood glucose level in both LDM and HDM rats; however, the hyperglycemia state persisted in these mercury exposed rats. The HDM rats treated with ALA behaved like the control above. The blood glucose levels at 30, 60, 90, 120 and 180 min were much lower than the corresponding HDM only rats.

**Lipid peroxidation:** As shown in Fig. 3, significant increases in MDA levels were observed in both LDM and HDM rats when compared with control rats. However lipid peroxidation as indexed by MDA level was significantly lower in the HDM-ALA rats compared with both LDM and HDM rats. ALA alone showed no difference when compared with the control and its values was significantly lower than those of LDM and HDM treated animals.

**Superoxide dismutase:** Figure 4 depicts the SOD activity in all the experimental groups. Significant decreases in SOD activity were observed in both LDM and HDM rats when compared with the control. However, co-treatment of ALA with HDM significantly improved and increased the SOD activity in the HDM-ALA rats.

**Reduced glutathione:** The activity of reduced glutathione in all the experimental groups was shown in Fig. 5. Activity of GSH was significantly reduced in both LDM and HDM rats when compared with the control. However, in the HDM-ALA rats, co-treatment of HDM with ALA significantly increased GSH activity when compared with HDM only rats.

**Catalase:** There was a significant decrease in the CAT activity in both LDM and HDM rats when compared with control rats (Fig. 6). Co-treatment of HDM with ALA however did not significantly increase the CAT activity in the HDM-ALA rats compared with both mercury treated groups of LDM and HDM. CAT activity in ALA rats was similar to the control values.

## DISCUSSION

This study was carried out to investigate the impact of exposure to mercury chloride on glucose homeostasis and the possible role of oxidative stress if any. In addition, the potential protective effects of Alpha Lipoic Acid (ALA), a naturally occurring and unique antioxidant on mercury chloride toxicity were investigated. The results obtained from the present study showed significant adverse changes in glucose tolerance and oxidative indices in mercury chloride exposed rats. Treatment with ALA however attenuated the mercury-triggered adverse changes.

The results obtained from this study showed that exposure to mercury chloride caused an increase in the fasting blood glucose level. The increase in blood glucose level may be due to mercury-induced decrease in glucose utilization. Moreover, the data obtained from the glucose tolerance test clearly indicate that blood glucose levels remain higher even after 180min of glucose load in mercury exposed rats. This suggests that mercury chloride has negative effects on glucose homeostasis possibly through inadequate glucose uptake, storage and disposal.

An impaired glucose tolerance state is considered as a transitional phase to the development of type 2 diabetes (Dhalla *et al.*, 2007); thus, exposure to mercury chloride appears to increase the risk of developing type 2 diabetes mellitus.

Oxidative stress is a crucial factor in the regulation of blood glucose level (Perez-Matute *et al.*, 2009). The data obtained from the present study indicated that mercury treatment promotes the development of oxidative stress. The increased lipid peroxidation level in the experimental rats may be due to mercury-induced imbalance in pro-oxidant and antioxidant system. This observation is consistent with previous reports in literature that suggest that mercury chloride increases lipid peroxidation in cells (Augusti *et al.*, 2008; Durak *et al.*, 2010). Lipid peroxidation is the process of oxidative degradation of poly unsaturated fatty acids (PUFA) and its occurrence in biological system can cause impaired glucose control (Rudich *et al.*, 1998; Ceriello *et al.*, 2000). Thus, it is plausible to speculate that exposure to mercury chloride may result in peroxidation of PUFA leading to the progressive loss of the pancreatic beta cells and ultimately results in hyperglycemia and glucose intolerance. Various studies have also suggested a strong relationship between increased lipid peroxidation level and glucose abnormalities (Brownlee, 2001; Rolo and Palmeira, 2006).

Antioxidant enzymes are critical part of cellular protection against reactive oxygen species and ultimately oxidative stress. Antioxidants involved in the elimination of ROS include SOD, CAT and GSH, respectively. The present study showed a decrease in the activity of all measured antioxidant enzymes in mercury-treated rats. The adverse decrease in antioxidant capacity vary in parallel with the degree of lipid peroxidation observed, since lipid peroxidation levels were significantly higher in mercury-exposed rat with lower antioxidant activities. The depressed activities of SOD, CAT and GSH observed in this study might support the hypothesis that mercury chloride-induced glucose abnormality is mediated by oxidative mechanisms. It is known that beta cells are particularly low in antioxidant enzymes particularly SOD, CAT and GSH (Perez-Matute *et al.*, 2009). The derangement in glucose homeostasis associated with mercury chloride may therefore be attributed to the ineffective scavenging of radicals which progressively increases the level of oxidants in the pancreatic beta cell leading to increased lipid peroxidation, and ultimately blunting insulin secretion. Furthermore, reduced antioxidant activity and/or increased oxidative stress may have triggered glucose abnormality. Increasing evidence abounds implicating oxidative stress as negative regulators of insulin signaling (Valko *et al.*, 2007; Bashan *et al.*, 2009). Therefore, inhibition of insulin signaling occasioned by mercury chloride-induced oxidative stress may result in inadequate glucose utilization leading to hyperglycemia and glucose intolerance.

If oxidative stress contributes significantly to the pathophysiology of a disease, then suppression of oxidative stress may be therapeutically beneficial. Many studies in literature have reported the protective effects of exogenously administered antioxidants, thus providing insight into the relationship between free radicals and diabetes (Mekinova *et al.*, 1995; Kim *et al.*, 2000; Borenshtein *et al.*, 2001; Maritim *et al.*, 2003). Treatment of mercury exposed rats with ALA improved glucose tolerance, reduced lipid peroxidation and increased antioxidant activities thereby indicating that oxidative stress was involved in the mercury induced hyperglycemia and glucose intolerance. The involvement of oxidative stress in the mechanism of mercury chloride-induced glucose abnormality is therefore buttressed by the results obtained from the mercury-exposed rats treated with ALA.

In conclusion, the results presented in this study clearly demonstrate the induction of glucose intolerance by mercury chloride and suggest the involvement of oxidative stress as an important regulator of glucose homeostasis during mercury chloride exposure. This mechanism of action is further buttressed by the attenuation of the mercury-triggered adverse changes by ALA. The detailed mechanism of mercury-induced glucose pathologies has not been fully elucidated and will be subject of future experiments.

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