Journal of Applied Pharmaceutical Science
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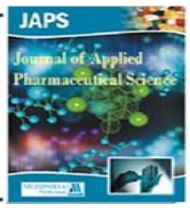

ISSN: 2231-3354
Received on: 20-11-2011
Revised on: 09:12:2011
Accepted on: 21-12-2011

Electrolytes alterations in plasma and urine after 28 days repeated oral dose toxicity of mercuric chloride in wistar rat

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ABSTRACT

An experiment was conducted to study the electrolytes alteration in experimentally induced mercuric chloride in wistar rat. For this rats were uniformly divided in four different dose group 0.0, 2.0, 4.0, and 8.0 mg/kg body weight ranging from asymptomatic to high dose for 28 consecutive days. In this experiment, blood was collected on 0, 14 and at the end of experiment. In mercuric chloride treated group dose dependent significant increase in plasma glucose, sodium, and chloride and creatinine level. Same parameters studied in urine showed significant increase in excretion of electrolytes and glucose in urine while urine creatinine was decrease. Mercuric chloride produced dose depended electrolytes alteration in wistar rat at given dose.

Keywords: Mercury, glucose, chloride, sodium, creatinine, serum, urine, rat.

INTRODUCTION

Development activities carry with, it the seeds of environmental damage, assisted and abetted by both needs and greed of man. Activities such as manufacturing, processing, transportation and consumption not only deplete the stock of natural resources but also add stress to the environmental system by accumulating the stock of waste. Mercury pollution is still a worldwide problem ever since the outbreak of mercury poisoning in Minamata, Japan way back in the 1950s and in Iraq in 1971-72 (WHO Report EHC-118, 1991). About 100 tonnes of organomercurials are produced in India every year (Annon, 1990). Moreover, recently certain common Indian food items like fish, prawn, cabbage and amaranthus have been found to contain high levels of Hg (Ghoshdastidar and Chakrabarti, 1991; Lenka et al.1992; Panda et al., 1992), Mercury accumulates in mammalian target organs and damages them (Macgregor and Clarkson, 1974). Only a few substances can reduce its toxicity (Vitamins D & E, thiol compounds, Se, Zn and Cu), and costly chelators like BAL and DMSA (dimercaptosuccinic acid) can mobilize it from the body (Megos and Webb, 1979). In general mercury toxicity derives from the fact that mercury binds to sulfide groups and disrupts the proper functioning of sulfhydryl enzymes. Three routes of exposure, toxicity, target organs and ultimately treatment strategies vary according to the species of mercury involved in the exposure. The kidneys excrete waste products of metabolism and play an important role in maintaining the homeostasis by regulating the body water and solute balance. In addition to the excretory function, the kidneys also have an endocrine function producing hormones like renin, erythropoietin etc. Inorganic mercury (HgCl2) has been shown to accumulate in kidneys4 along with in other organs. A specific concern associated with mercury exposure in humans is the need for effective therapy in dealing with intoxication. In this respect, chelation

therapy is the most commonly used and seen as the least invasive. 5 Chelating agents compete with the in vivo binding site for the metal ion through the process of ligand exchange. 6 The toxic metal bound to the chelating agent is excreted from the body through the urine or feces. Among chelating agents currently available, the sodium salt of 2,3-dimercapto-1-propane-sulfonate (DMPS) has been found to be highly effective, particularly with respect to promoting mercury elimination following,

MATERIALS AND METHODS

Fourty Wistar rats of 5-7 weeks old were obtained from the Zydus Research centre, Zydus pharmaceutical company (Ahmedabad- India). The animals were housed in Polypropylene cages (5 animals /cage) and received water and pelleted food ad libitum. All rats were kept under controlled conditions of temperature (22±3ºC) and humidity (60±5%). They were given pellet food (Amrut feeds Ltd., Pune, India) and drinking water ad libitum. A twelve hour day and night cycle was maintained in the animal house. The experimental protocol met the national guidelines (CPCSEA) on the proper care and use of animals in the laboratory research. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

Chemical

Mercury was obtained in the form of Mercuric chloride from Merck India Ltd. (Mumbai India), and dissolved in the distilled water and administered through oral gavage.

Experimental Design

Experiment was conducted for four weeks. The animals were divided into following groups.

Group I (n = 10) - Controlled animals (treated with vehicle alone), Group II (n = 10) - 2.0 mg/Kg HgCl2, Group III (n = 10) - 4.0 mg/Kg HgCl2, Group IV (n = 10) - 8.0 mg/Kg $HgCl_2$.

Blood samples were collected on 0, 14 and 28 day of experiment. Blood was collected similar time of day and by retro-orbital venous plexus puncture under ethyl- ether anesthesia. Urine samples were collected on dry ice, centrifuged and stored at -10 °C till assayed.

Biochemical and Urine analysis

All the biochemical parameters viz. Glucose, sodium, creatinine and chloride were analyzed by using respective Accucare Diagnostic Kit (LAB-CARE Diagnostic India Pvt. Ltd., Mumbai).

RESULTS

The serum analyzed concentrations in the two groups are shown in **Table 1.** The significant difference was observed for creatinine concentration between the two groups (treatment and control) after 4 weeks of the experiment. Mean serum sodium and chloride concentrations were significantly higher in rats treated with mercury compared to the control rats. Blood glucose level was significantly higher in the exposed rats.

The effect of mercuric chloride on the excretion of urinary electrolytes is dose dependent (**Table 2**). The dose of 8.0 mg/kg significantly increased the excretion of all the electrolytes estimated in this study. The tested metal at 4.0 mg/kg still cause significant saluresis and kaliuresis (P<0.05) when compared to control. However, the tested metal at dose of 2.0 mg/kg did not bring about any significant increase in the electrolytes excretion.

A significant (P < 0.05) decrease in urinary creatinine was observed in all treatment groups on 14th and 28th day post-treatment, except Group-II on 14th day. While significant (P < 0.05) increased in urinary glucose excretion was reported only in Groups-III and IV at 28th day post-treatment with mercuric chloride as compare to control.

Table 1. Comparison of Mean (± S.E.) plasma biochemical electrolytes in rats of different experimental groups at one to four weeks of post-treatment.

Groups		Week Intervals		
		0 day	14 day	28 day
Glucose mg/dl	I	98.66 ± 2.49	107.28 ± 1.96^{a}	108.75 ± 2.34^{b}
	II	99.33 ± 2.50	108.17 ± 2.02^{a}	112.22 ± 2.63^{ab}
	III	99.36 ± 2.74	111.46 ± 1.93^{a}	115.30 ± 2.12^{a}
	IV	99.47 ± 3.15	113.46 ± 2.49^{a}	116.23 ± 2.34^{a}
Sodium mEq/l	I	147.12 ± 1.04	148.87 ± 0.78^{d}	148.48 ± 0.94^{d}
	II	147.56 ± 0.81	150.82 ± 1.90^{cd}	161.76 ± 2.46^{c}
	III	148.09 ± 0.72	159.48 ± 2.80^{b}	171.06 ± 5.06^{bc}
	IV	148.34 ± 1.74	168.79 ± 4.25^{a}	184.91 ± 3.09^{a}
Chloride mEq/l	I	108.49 ± 0.63	107.53 ± 1.55^{b}	109.58 ± 1.98^{d}
	II	107.10 ± 0.93	108.38 ± 2.02^{b}	119.81 ± 3.31^{c}
	III	106.63 ± 1.23	110.57 ± 1.88^{b}	133.35 ± 2.44^{a}
	IV	105.46 ± 2.13	119.34 ± 3.68^{a}	129.42 ± 2.10^{ab}
Creatinine mg/dl	I	0.36 ± 0.0	0.39 ± 0.01^{bc}	0.40 ± 0.0^{c}
	II	0.37 ± 0.0	0.38 ± 0.01^{c}	0.51 ± 0.04^{bc}
	III	0.36 ± 0.0	0.38 ± 0.01^{c}	0.95 ± 0.11^{a}
	IV	0.37 ± 0.01	0.63 ± 0.02^{a}	1.06 ± 0.05^{a}

Note : Superscripts are to be read column wise for mean comparison. Mean with similar superscripts in column do not differ significantly (P < 0.05).

Table 2. Comparison of Mean (± S.E.) Urine biochemical electrolytes in rats of different experimental groups at one to four weeks of post-treatment.

Groups		Week Intervals		
		0 day	14 day	28 day
Glucose mg/dl	I	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.00
	II	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.00
	III	0.0 ± 0.0	0.0 ± 0.0	12.50 ± 0.50^{b}
	IV	0.0 ± 0.0	0.0 ± 0.0	15.21 ± 1.52^{a}
Sodium mEq/l	I	153.01 ± 1.15	153.30 ± 1.37	155.61 ± 1.27^{d}
	II	153.38 ± 1.29	155.84 ± 1.74	170.15 ± 2.78^{c}
	III	153.22 ± 1.24	157.06 ± 1.47	174.39 ± 4.13^{bc}
	IV	153.53 ± 1.21	157.99 ± 1.37	187.05 ± 4.10^{a}
Chloride mEq/l	I	62.94 ± 0.70	69.69 ± 0.81^{b}	71.16 ± 0.96^{d}
	II	63.54 ± 0.86	74.46 ± 1.27^{a}	80.44 ± 1.48^{c}
	III	63.42 ± 0.75	75.18 ± 1.33^{a}	85.60 ± 1.47^{b}
	IV	63.85 ± 0.80	76.42 ± 1.62^{a}	90.04 ± 1.03^{a}
Creatinine mg/dl	I	33.56 ± 1.21	32.03 ± 1.28^{a}	31.95 ± 1.17^{a}
	II	33.25 ± 1.13	31.82 ± 1.31^{a}	25.35 ± 1.17^{b}
	III	33.53 ± 1.26	28.00 ± 1.89^{b}	21.91 ± 0.84^{d}
	IV	33.25 ± 1.12	26.78 ± 1.74^{bc}	17.94 ± 1.43^{d}

Note : Superscripts are to be read column wise for mean comparison. Mean with similar superscripts in column do not differ significantly (P < 0.05).

DISCUSSION

The present investigation was carried out in order to determine, the biochemical repercussions of daily HgCl2

administration in male Wistar rats from serum and Urine. Indeed, we revealed here that continuous oral administration of HgCl2 during 14, and 28 days had a great impact on the measured biochemical parameters, by which mainly electrolytes position were evaluated.

The toxicity of mercury depends on its chemical form. Various mercury compounds have different toxicities depending on physical and chemical properties that affect absorption, distribution, tissue affinities and stability within the biosystem. For instance, elemental mercury in the liquid state has unique toxic effects that differ from those of mercury vapour; likewise, organic mercury molecules are toxicologically different from inorganic forms (NTP, 1993). HgCl2 is an inorganic compound used in various fields. Its numerous effects were evaluated in many toxicity and carcinogenicity studies because of its extensive use and its wide occurrence as an environmental pollutant (NTP, 1993). Once absorbed, HgCl2 is distributed in all tissues and low fractions have been shown to easily cross the brain-blood barrier and the placenta. However, the kidney was considered as the primary target organ, in which HgCl2 is intensively accumulated following chronic exposure (WHO, 1996).

The hyperglycaemia has been linked to oxidative damage to cell (Mohamed *et al.,* 1999). One possible mechanism for hyperglycaemic-induced oxidative stress involves auto-oxidation of glucose, which can result in the production of O_2– and other ROS. Another way for hyperglycaemia may be due to enhanced gluconeogenesis and glycogenolysis and decreased glucose utilization under oxidative stress enzyme produced by mercury. But for glucose in Urine, it is freely filtered by the glomeruli with a fractional excretion of less than 0.1 per cent in human being. Reabsorption of glucose occurs predominantly on the brush border membrane of the convoluted segment of the proximal tubule glucose enter the tubular cells by an active carrier mediated transport process, which is sodium dependent and exists *via* the basolateral membrane by facilitated diffusion by a glucose transported, which is sodium independent. In other way, if the glucose level exceed more then its threshold value. Kidney became unable to reabsorb such much amount of glucose and excreted in urine. But in the present study the hyperglycaemia was not at threshold level. But hyperglycaemia with tubular/nephrone degeneration and disruption in sodium levels which were authenticated by histopathological examination of kidney **(**Sheikh et al., 2011).

A significant ($P < 0.05$) increased in serum creatinine mostly at the dose rate of 4 mg/kg and 8 mg/kg of body weight was observed during this experiment. The present finding was supported by Joshua *et al*. (2007) and Gray and Kavlock (1987) in rat.

Serum creatinine concentration is one of the traditional screening indices for kidney function and renal structural integrity. In the present, the increase in creatinine might be due to renal damage. The observed increase in plasma and urine creatinine level is therefore a likely indication of glomerular dysfunction in rats exposed to mercuric chloride 28 days (Joshua *et al.* 2007 and Gray and Kavlock 1987).

The decreases in urinary creatinine level were also reported by Carmingnani *et al.* (1992) in rat treated with methyl mercury @ 0.2 mg/ml for 180 days and Joshua *et al.* (2007). The decrease in urinary creatinine excretion was also supported by Nicholson *et al.* (1985).

Creatinine is used to evaluate GFR because; it is almost exclusively cleared by glomerular filtration and is neither secreted nor absorbed by the renal tubules. Creatinine is not significantly metabolized or excreted anywhere-else in the body (insignificant but amount excreted in sweat and faeces). Creatinine clearance provides a reasonable estimation of glomerular filtration rate in domestic animals, but is not equivalent to glomerular filtration rate (Kaneko *et al.*, 1997), which occurred in mercury toxicity due to damage of glomeruli and tubules.

The increase in serum sodium may be due to decrease thirst that causes the hemoconcentration and dehydration further more by cerebral damage which causes interference in ADH. Secretion, this inhibition of ADH secretion, this may result in loss of large volumes of dilute urine and consequent rise in plasma osmolality and serum sodium concentration (Zalups 1995, Endo *et al.* 2003). Or in other way the increased in excretion of sodium and chloride ions, this may be due to the inhibitive effect on tubular cells reabsorption of these ion and the inhibition in reabsorption might be due to extensive damage produce by very toxic heavy metal *i.e.,* mercury (Daston *et al.* 1984).

In conclusion, our findings suggest that exposure to HgCl2 in wistar rats has differently and chronologically affected the levels of glucose, sodium, chloride and creatinine in plasma and urine.

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