EFFECT OF MERCURIC CHLORIDE INDUCED TOXICITY ON BIOCHEMICAL CHANGES IN CHICKEN (KADAKNATH)

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ABSTRACT

The Present Study on the effect of mercuric chloride toxicity on biochemical changes in chicken showed that the serum biomarker enzymes (AST, ALT, ALP, GGT) and biomolecules glucose, total bilirubin creatinine, urea, BUN were increased significantly in both the treatment groups as compared to the control group. At the same time significant reduction in concentration of total plasma protein and serum albumin was observed.

KEW WORDS : Mercuric chloride , toxicity , chicken , biochemical changes

INTRODUCTION

Mercury is a naturally occurring element and it is used in a wide variety of products such as fungicides, seed dressing agents in agriculture, antiseptics and diuretics. Both forms of mercury, organic and inorganic, banish as affluent from industrial processes and are converted into soluble methyl mercury in the lakes and rivers which ultimately mix with sea water, where it is taken by living organisms, notably fishes and produce deleterious effects (Garg, 2004). General exposure to such biologically-active chemical agent has been shown to exacerbate through contaminated water and food (Magos and Clarkson, 2006).

Mercury (Hg) being highly toxic metal that results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive and developmental disorders (Risher and Amler, 2005). Kidneys, liver and gastrointestinal tract are mainly targeted by inorganic Hg compounds, such as mercuric chloride (Schurz *et al.*, 2000; Ghosh and Sil, 2008). The oxidative stress was strongly suggested as one of the crucial mechanisms in Hg-induced pathological aspects (Lund *et al.*, 1993; Clarkson, 1997; Perottoni *et al.,* 2004). However, biochemical parameters are still more indicative of early pathological changes following subchronic and chronic Hg exposure (Wadaan, 2009). Thus, the purpose of the present study was to evaluate biochemical changes during $HgCl_2$ intoxication and to examine how the chicken responds chronically to this pollutant. Initially, $HgCl_2$ induced hepatoxicity, nephrotoxicity and metabolic disturbances were estimated by determining the various enzymes and biomolecules in serum.

MATERIALS AND METHODS

A total of 36 healthy one week old kadaknath (*Jet black, Pencilled*) chicks of either sex were obtained from A.I.C.R.P. on Poultry, J.N.K.V.V., Jabalpur and used for the experiment . The birds were maintained under identical hygienic conditions and fed on the standard poultry feed supplied by the Poultry Project, Jabalpur. Mercuric chloride powder ($HgCl_2$, Prod. No. 25284, Batch No.NL1677 6408 s) manufactured by Qualigens Fine Chemicals, Mumbai was administered with feed according to the design of the experiment.

The birds were randomly distributed into 3 groups, 12 birds in each group. Two experimental groups (Gr. II and III) were treated with $HgCl_2$ by mixing with feed @ 50 ppm and 100 ppm which was given orally daily for 21 and 42 days and the control group (Gr.I) was fed with standard feed.

At the end of each period of treatment, six birds from each group were sacrificed by decapitation and the blood was then collected in tubes and serum was separated by routine procedure.

Serum Alanine aminotransferase (ALT) (IFCC Method), Aspartate aminotransferase (AST) (IFCC Method), Alkaline phosphatase (ALP) (Lowry's Method), Gamma glutamyl transferase (GGT) (IFCC Method), Total bilirubin (Diazo Method), Glucose (Trinder's Method), Total plasma protein (Biuret Method), Serum protein (Biuret Method), Serum albumin (BCG Dye Method), Urea (GLDH - Urease Method), Blood urea nitrogen (BUN) (GLDH - Urease Method), Creatinine (Jaffes's Method).

All these determinations were carried out using standard commercial kits using Erba , CHEM -5 Plus V2 semi auto analyzer (Mannheim, Transasia Bio-medicals Limited . The manufacturer's instructions on the assay procedures were strictly followed.

The analysis was realized using CRD by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The serum biomarker enzymes (AST, ALT, ALP, GGT) and biomolecules glucose, total bilirubin creatinine, urea, BUN were increased significantly in both the treatment groups as compared to the control group. At the same time significant reduction in concentration of total plasma protein and serum albumin was observed (Table 1).

The toxicity of mercury depends on its chemical form. Various mercury compounds produce different types of toxicity depending on physical and chemical properties that affect absorption, distribution, tissue affinities and stability within the biosystem.

$HgCl_2$ 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; Langford and Ferner, 1999). Once absorbed, $HgCl_2$ 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 $HgCl_2$ is intensively accumulated following chronic exposure.

The present investigation was carried out in order to determine, in a chronological manner, the biochemical repercussions of daily $HgCl_2$ administration in chicken. Indeed, we revealed here that continuous oral administration of $HgCl_2$ during 21 and 42 days had a great impact on the measured biochemical parameters, by which mainly hepatotoxicity and nephrotoxicity are evaluated.

Plasma proteins play a prominent role in preventing increase of systemic metal-related free fractions, thus preserving the physiological functions by delaying metal accumulation and toxicity. In this study, plasma proteins levels were significantly reduced after 21 and 42 days of $HgCl_2$ exposure. The alkaline phosphatase (ALP) is a well-known indicator of multiple toxicity cases, including those related to hepatic and renal dysfunctions. This enzymatic parameter is widely thought to be one of the most sensitive markers of Hg toxicity (Kumar *et al*., 2005). In the present study significant increase was obsereved in serum ALP activity after 21 and 42 days of daily $HgCl_2$ exposure . ALP is mainly a liver cytoplasmic enzyme; the elevation in its activity in serum is generally related to necrotic lesions in this organ (El-Shenawy and Hassan, 2008). In this respect, ALP activity alterations may result from Hg effect primarily on hepatic and renal tissues. Furthermore, mercury may affect intestinal functions during absorption processes, thus inhibiting the enzyme synthesis and activity. Simultaneously, plasma urea concentration revealed a significant increase during the whole chronology of experiment. This result is undoubtedly related to acute and persistent renal injuries, thus confirming that the kidneys are very sensitive to Hg exposition (Agarwal and Behari, 2007). When attached together, our findings suggest that $HgCl_2$ -exposed chickens had very serious renal disturbances. This $HgCl_2$ -induced nephropathy would be associated with glucose reabsorption

Table 1: Mean values of biochemical parameters in Kadaknath chicken fed with Mercury Chloride

Parameters	Day 21			Day 42		
	Group I	Group II	Group III	Group I	Group II	Group III
AST	161^{c}± 13.34	242^{b}±13.23	295^{a}± 13.16	156^{d}± 13.26	250^{b}± 13.86	301^{a}± 13.45
ALT	37.00^{c} ± 2.79	47.00^{b} ±2.68	61.00^{a} ± 2.38	38.82^{c} ± 3.24	51.26^{ab} ± 3.21	60.65^{a}± 3.26
ALP	512.00^{d}± 3.76	551.33^{b}± 3.56	590.66^{a}± 3.45	570.00^{e}± 3.56	602.00^{cd}± 3.45	655.00^{a}± 3.86
GGT	8.60^{c}± 0.54	9.70^{c}± 0.65	14.26^{a}± 0.53	9.12^{b} ± 0.50	12.89^{a}± 0.49	14.29^{a}± 0.54
Total Bilirubin	1.00^{d} ± 0.15	2.18^{ab} ±0.14	2.28^{a}± 0.12	1.00^{d} ± 0.11	2.30^{ab}± 0.16	2.50^{a} ± 0.18
Glucose	230.00^{c}± 11.56	315.00^{ab} ±11.45	332.00^{a}± 11.56	235.83^{b}± 11.48	317.00^{a}± 13.45	331.00^{a}± 13.52
TPP	5.90^{a}± 0.18	5.70^{ab}± 0.17	5.40^{bv}± 0.14	6.65^{a}± 0.18	6.40^{a}± 0.16	6.30^{a} ±0.19
Serum Protein	4.15^{a}± 0.17	3.21^{bc}±0.16	2.98^{d}±0.15	4.60^{a}±0.14	4.43^{a}±0.19	4.18^{a}± 0.20
Serum Albumin	2.03^{ab}± 0.07	1.95^{ab}± 0.08	1.83^{b}± 0.05	1.86^{ab}± 0.03	1.97^{ab}± 0.05	2.01^{a}± 0.08
Urea	6.00^{d}± 0.38	8.00^{c}± 0.31	10.80^{a}± 0.32	6.20^{d}± 0.30	8.12^{bc}± 0.28	10.41^{a}± 0.36
BUN	2.82^{d} ± 0.09	3.76^{c} ± 0.08	5.01^{a}± 0.04	2.92^{d}± 0.06	3.78^{bc} ± 0.04	4.86^{a}± 0.04
Creatinine	0.50^{a}± 0.12	0.68^{a}± 0.13	0.85^{a}± 0.15	0.50^{b}± 018	0.88^{ab}± 0.11	1.16^{a}± 0.17

***Note**: Mean values bearing common superscripts within columns (between groups) did not differ significantly (P e"0.05).*

and urea excretion alterations, leading to uremia as well as to hypoproteinemia (due to proteinuria) that indicates glomerular damage (Al-Madani *et al*., 2009). Additionally, this early nephrotoxicity being severe and irreversible, as revealed by highly persistent uremia, was accompanied by elevated ALP levels at day 21 and 42.

Mercury contamination underlie the serious imbalance in the oxidative/antioxidant ratio. An increase in reactive oxygen species (ROS) formation by $HgCl_2$ may induce liver cell membrane structural and functional alterations, leading to noxious metabolic outcomes (Sharma *et al*., 2007). Furthermore, acute administration of $HgCl_2$ causes toxic effects on kidney and liver tissues and this damage was associated with the increase in serum ALP activity and urea (Ghosh and Sil, 2008).

Our findings suggest that exposure to $HgCl_2$ in chickens has differently and chronologically affected the plasma levels of transminases , ALP, proteins, urea, bilirubin , creatinine and plasma glucose. Interestingly, the accelerated increase in urea concentration suggest that $HgCl_2$ is primarily a nephrotoxic, thus highlighting the fact that general toxicological directives should basically focus on renal function during the early stages of $HgCl_2$ contamination, because early $HgCl_2$ - induced nephrotoxicity could exacerbate the biochemical imbalance and accelerate hepatotoxicity. Transaminases play an important role in carbohydrate and amino acid metabolism in the tissues of fish and other organisms . Alanine aminotransferase is a key metabolic enzyme released on the damage of hepatocytes. The enzyme shows a increasing level in serum at 21 and 42 days administration of HgCl2 , indicating its adaptive response to its leakage into the blood stream due to the metal toxicity. Also, the alanine aminotransferase has a part in transforming protein to glycogen, which is the major reserve fuel of the body during the stress-induced toxicity in the liver. This result is in accordance with the results of previous investigators on fresh water fish. The results indicate that under the influence of different heavy metals or in a state of stress, the damage of tissues and organs may occur with concomitant elevation and liberation of transaminases into the circulation. The aspartate aminotransferase belongs to the plasma non-functional enzymes which are normally localised within the liver cells, heart, gills, kidneys, muscle and other organs. Monitoring of liver enzyme leakage into the blood has proved to be a very useful tool for toxicity studies in the liver.

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