Ameliorating Effect of NaMSA on HgCl₂ Induced Hepatogenous Poisoning in Albino Rats

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ABSTRACT
Heavy metals are free radicals contains one or more unpaired electrons produces superoxide anion, interact with other molecules to generate secondary ROS, either directly or prevalently through enzymes or metal catalyzed process and induces severe alterations in the tissues of both animals and humans. The aim of the present investigation was to detoxify mercuric chloride induced hepatotoxicity using morin-5'-sulfonic acid sodium salt and determining the optimum dose for the same. Administration of mercuric chloride (1.25mg /kg) capable of causing the Hepatotoxicity through the elevation of AST, ALT, ALP, GGT, bilirubin and decreasing of total protein, at the same time when the morin was simultaneously administered in the form of morin-5'-sulfonic acid sodium salt in 3 different doses (25 mg/kg, 50 mg/kg and 100 mg/kg) with mercuric chloride (1.25 mg/kg b.wt), all the 3 doses showed protective effect on mercury induced toxicity among them 50 mg / kg body weight was found to be more effective than remaining doses hence the optimum dose for morin-5'-sulfonic acid sodium salt fixed to be 50 mg/kg body weight against mercuric chloride (1.25mg /kg body weight, p.o). On the basis of the obtained results, it is concluded that morin in the form of morin-5'-sulfonic acid sodium salt has more protective effect against mercuric chloride induced poisoning in rats, due to its free radical scavenging ability induces enzymatic and non-enzymatic antioxidants and chelates free mercuric ions. The mechanism of detoxifying action of morin-5'-sulfonic acid sodium salt is most probably based on their high affinity and ability to form insoluble complexes with mercury ions, which will get excreted in faeces and urine.

Key words: Morin-5'-sulfonic acid sodium salt (NaMSA), Mercuric chloride (HgCl₂), Hepatotoxicity and Aspartate transaminase (AST).

1. INTRODUCTION
Heavy metals are tracing metals with a density 5 times more than that of water [1]. Most common and harmful heavy metals are aluminium (Al), arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg) and nickel (Ni). Both occupationally and environmentally these heavy metals constitute a significant potential threat to human health [2].

Heavy metals are free radicals contains one or more unpaired electrons [3] acts in many ways through the production of superoxide anion, the primary ROS and can further interact with other molecules to generate secondary ROS, either directly or prevalently through enzymes or metal catalyzed process [4].

1.1 Sources of Mercury
Mercury occurs in 3 forms i) Elemental mercuric (ii) Inorganic mercuric and iii) Organic mercuric. Mercuric poisoning can be caused by any number of methods of exposure. i) Amalgam dental fillings are a main cause ii) Eating fish that have been exposed to mercury in the environment, industrial and work place; iii) Exposures such as those in the paint industry. The methyl mercury concentration ranges from 20.4 to 344.4 ng/g dry weight and maximum concentration has been found in crabs and prawns [5].

1.2 Mercury risk in India
The concentration of mercury in fish in other sea food consumed in certain coastal areas reported in the range of 0.03-10.82 g/g compared to the permissible limit of 0.5 g/g. There is a potential risk to human health and environment due to the entry of mercury in food chain. The basket fruits and vegetables contain several folds higher concentration of mercury in certain industrial area against prescribed Indian standards. The overall total mercury concentration ranged from 62.5 to 548 ng/gm (189 ng/gm)\textsuperscript{[15]}. The bureau of India standards (BIS) has laid down safety limits for drinking water at 0.001 mg of mercury per liter. A number of samples of groundwater in some industrial belts have shown concentrations of mercury higher than safe standards.

1.3 Mechanism

Multiple mechanisms have been proposed to explain the biological toxicity of HgCl\textsubscript{2} by investigating the biochemical fate of various Hg forms\textsuperscript{[6]}. Indeed, the Hg\textsuperscript{2+} form has shown a great affinity for endogenous biomolecules-associated with thiol (-SH) group\textsuperscript{[7]} the oxidative stress was strongly suggested as one of the crucial mechanisms in Hg-induced pathological aspects\textsuperscript{[8, 7 & 9]}. However, biochemical parameters are still more indicative of early physiological changes following sub chronic and chronic Hg exposure\textsuperscript{[10]}. Studies indicate that transition metals act as catalyst in the oxidative reactions of biological macromolecules; therefore the toxicities associated with these metals might be due to oxidative tissue damage.

1.4 Toxic effects

Hg is a divalent metal without any biological functions. It is a widespread environmental and industrial pollutant induces severe alterations in the tissues of both animals and humans. Parental administration of mercuric chloride (HgCl\textsubscript{2}) result in the lipid per oxidation occurs in kidney, liver and other tissue of rats and mice. It primarily affect the CNS, liver and renal system. Mercuric poisoning symptoms are systemic. This means that it doesn’t just affect one part of the body, but it affects every system in the body. In addition to this, mercuric poisoning inhibits the immune system and therefore the patient will have other diseases as well\textsuperscript{[11]}.\textsuperscript{[12-14]} also known to form complex with specific enzyme inhibitions and at the intracellular level likely enhance the blood brain barrier.

1.5 Mercury and Liver damage

Organic Hg compounds specifically affects the central nervous system\textsuperscript{[15]}, while kidneys, liver and gastrointestinal tract are mainly targeted by inorganic Hg compounds, such as mercuric chloride (HgCl\textsubscript{2})\textsuperscript{[13 & 14]}.

An increase in reactive oxygen species (ROS) formation by HgCl\textsubscript{2} may induce liver cell membrane structural alterations\textsuperscript{[16]} showing symptoms of total mercuric load.\textsuperscript{[12]}. In most cases the final major toxic form of mercury found in the affected tissues and blood\textsuperscript{[17]}.

1.6 Chelating Agents

Chelating agents are primarily sulphydryl-containing compounds such as mono- or dithiol molecules. At the molecular level, the chelation process appears as an inevitable tug of war between the chelating agents and the competing biological ligands\textsuperscript{[18]}. Many chelating therapeutic agents available in practice for acute inorganic mercuric poisoning with the following efficiency DMPS> DMSA> penicillamine > ALA > EDTA removed 20 percent. Glutathione and N-acetylcysteine (NAC) are recommended by some physicians but have been shown to increase mercury concentrations in the kidneys and the brain (Rooney, 2007). Drugs such as DMP not effectively cross the cell membrane nor the blood brain barrier\textsuperscript{[19]}, Use 20% temporarily\textsuperscript{[12]}. This indicates a much larger body storage of binding compounds have been reported to redistribute mercury into the kidney.

1.7 Flavonoids

Due to the limitations of existing chelating agents either in terms of efficacy/safety, it is necessary to find a new active component with promising approach to protect mercury induced toxicity. Currently flavonoids are being emerging topic which has more beneficial activity. Interactions of flavonoids with metal ions can lead to chelate formation. The chelation of metals is crucial in the prevention of radical generation, which damage target biomolecules. Moreover, using natural chelators such as flavonoids are better than the synthetic ones due their toxicity effects. Morin hydrate is a flavonoid with antioxidant properties. It has been shown to protect cells against oxygen radical damage in vitro\textsuperscript{[20]}. Morin is insoluble in water hence, it was converted to soluble form through sulphonation and formed Morin-5’-sulfonic acid sodium salt (NaMSA).

Morin-5’sulfonic acid sodium salt (NaMSA) is easily soluble in water and keep the properties of the parent compounds. The aqueous solubility of...
NaMSA under the same conditions was 2.7 \times 10^{-2} \text{ mol/dm}^3. \text{ Sulfonic quercetin and morin derivatives can be considered to be multi protonic acids, which dissociate in aqueous solutions yielding respective anions. This has the sulfonic Morin derivative NaMSA was used as antioxidants. NaMSA is characterized by low toxicity to laboratory animals (mice and rats) [21, 23].}

2. MATERIALS AND METHODS

2.1 Chemicals used

The fine chemicals Alanine, Aspartic acid, Cholesterol, 2,4 dinitrophenyl hydrazine, DAM-TSC reagent, Phosphotungastic acid, Sodium pyruvate, Urea, Sulphuric acid, Albumin, Total bilirubin used for the present study purchased from Merck companies (AR grade). Mercuric chloride, Morin obtained from Sigma Aldrich., Bangalore, India.

2.2 Methods

Standard methods were used for the estimation of factors, activities of Aspartate transaminase (AST; E.C.2.6.1.1) and Alanine transaminase (ALT; EC. 2.6.1.2, by modified Reitmann frankel method. The activity of GGT (E.C.2.3.2.1) by the method of Lie Wen et al: Kopacz, 2003.

2.3 Animals

Wister strain albino rats weighing 180-220g were used. The animals were housed in spacious cages under hygienic condition and maintained on commercial diet. It was supplied by the “Hindustan Lever limited”, Mumbai, under the trade name “Gold mohur Feeds”. Water was provided at libitum. The rats were acclimatized in animal house for ten days before starting the experiment. This study was approved by CPCSEA, New Delhi and Institutional Ethical Committee of Adhiparasakthi College of Arts and Science. No. APCS/IAEC/2010/01.

2.4 Preparation of HgCl\textsubscript{2} and induction of multiple organ failure

Rats administered orally by stomach tube with mercury in the form of mercuric chloride (1.25 mg/kg b.wt \textsuperscript{[23]} dissolved.

2.5 Preparation of Morin-5’-Sulfonic Acid Sodium Salt

Morin was purchased from sigma chemicals, USA is not soluble in water but soluble in alcohol 50 mg/ml.so, it is necessary to convert insoluble form of morin to water soluble Morin-5’-Sulfonic Acid Sodium Salt .hence, sulphonation reaction was carried out and morin was converted as Morin-5- Sulfonic acid Sodium Salt \textsuperscript{[21]} and used for the present experiment.

2.6 Synthesis of NaMSA (Morin- 5’- sulfonic acid sodium salt)

Morin is converted into morin- 5’- sulfonic acid sodium salt according to the method of Lie Wen et al: Kopacz, 2003.

A total number of 36 rats were taken for this present study while selecting the rats considering carefully the age, sex (male) and weight of each rat.

Group A: (control): Rats orally administrated with 0.9% saline for 30 days.

Group B: Rats intraperitonially administered with morin-5’-sulfonic acid sodium salt dissolved in water (100 mg/kg., p.o) for 30 days.

Group C: Rats administered orally by stomach tube with mercuric chloride (1.25 mg/kg) dissolved in 0.9 % saline for continuous 30 days. The dosage of mercuric chloride determined from Elizabeth et al., (2001).

Group D: Rats orally administrated with mercuric chloride (1.25mg/kg body weight) dissolved in 0.9% saline for continuous 30 days followed by morin-5’-sulfonic acid sodium salt dissolved in water (25mg/kg., p.o) simultaneously for 30 days.

Group E: Rats orally administrated with Mercuric chloride (1.25 mg/kg body weight) dissolved in 0.9% saline for continuous 30 days followed by morin-5’-sulfonic acid sodium salt dissolved in water (50mg/kg.,p.o) simultaneously for 30 days.

Group F: Rats orally administrated with Mercuric chloride (1.25 mg/kg body weight) dissolved in 0.9% saline for continuous 30 days followed by morin-5’-sulfonic acid sodium salt dissolved in water (100mg/kg., p.o) simultaneously for 30 days.

2.7 Preparation of Serum and Plasma

At the end of the experimental period (30 days), all the animals were anesthetized with intramuscular injection of ketamine (75mg/kg b.wt) and sacrificed by cervical decapitation. Blood was collected and centrifuged for serum separation. Blood was collected with anticoagulant and centrifuged (2000 x g for 20 min) to separate plasma. The tissues were dissected out, weighed, minced and homogenized (10% w/v) in Tris-HCL buffer (0.1 M; pH 7.4) and centrifuged at 3000 x g for 20 min at 4°C. The resulting supernatant was used for the analysis.

2.8 Statistical Analysis

The values were expressed as mean value (n=6) of + S.E.M, The \textit{in vivo} experimental data were
analyzed using one way analysis of variance by the Duncan’s Multiple comparison test to determine the level of significance p<0.05 was considered as statistically significance.

3. RESULTS

3.1. Effect of NaMSA on Mercuric Chloride induced changes in AST, ALT and ALP activities

Administration of mercury in the form of mercuric chloride (HgCl2) elevated the serum hepatic markers AST, ALT, ALP compared with control value significantly (0.05) whereas the trend was opposite in the simultaneous administration of HgCl2 and NaMSA that is the AST, ALT, ALP levels remains closure to the normal (Figure 1).

3.2. Influence of NaMSA on Mercuric Chloride induced changes in GGT activity

Mercuric chloride is one of the industrial pollutants known for its impact on induction of severe alteration in the tissue of both humans and animals (Figure 2).

Simultaneous administration of NaMSA and HgCl2 brought towards normal which was elevated from the normal during the administration mercuric chloride significantly (p < 0.005).

3.3. Changes in the level of bilirubin upon NaMSA and mercuric chloride

Bilirubin is the end product of hemoglobin metabolism, due to the oxidative stress induced by HgCl2 affected the life span of RBC and the bilirubin formation increased significantly (p < 0.005) with respect to the control rats. Whereas, the stress was declined and bilirubin value returned to the normal level upon the administration of NaMSA with HgCl2 (Figure 3).

3.4. Effect of NaMSA on HgCl2 induced changes in the level of protein of control experimental rats

Protein is one of the very good markers for reflecting the status of the cell. The necrosis occurred due to mercuric chloride induced oxidative stress decreased the protein synthesis and the level shows low in the plasma significantly (p < 0.005). The trend was different and the protein level is similar to the control rats. The significant (p < 0.005) retain of protein could be the chelating effect of NaMSA (Figure 4).

4. DISCUSSION

Hepatitis is a major public health problem worldwide, responsible for considerable morbidity and mortality from chronic liver disease. Developing countries like India and others have struggled to manage the impact of hepatitis along with the growing burden of obesity, type II diabetes, hypertension and coronary heart disease.

Enzyme markers are better indicators of the status of the organ in which it is present. The Cardiac markers LDH, CPK also showed significant elevation (P < 0.05) in the HgCl2 administered rats. But NaMSA and HgCl2 administration did not influence much on the LDH and CPK level, hence they remains almost similar to value of control.

Findings which showed increased activity of AST, ALT, and ALP upon HgCl2 administration, it is supported by [27,28] observation, HgCl2 intoxication causes a significant elevation of AST. According to Rao and [29] observation mercury in the form of mercuric chloride Significantly reduced the body weight of rats, whereas ALP and ACP activity were increased.

Hepatic functions, synthetic ability and the integrity of hepatocytes were affected upon the deleterious effects of mercury and reflected in the elevated levels of ALT and AST activities whereas [28,30] reported both acute and chronic exposure to mercury was attributed to its pathological effect in hepatic tissue.

The alkaline phosphatase (ALP) is a well-known indicator of multiple toxicity cases, including those related to hepatic and renal dysfunctions and being widely thought to be one of the most sensitive markers of Hg toxicity.

Total Plasma protein is another marker levels were significantly reduced due to HgCl2 toxicity (Kumar et al., 2005) and HgCl2 Exposure affected the plasma levels of proteins. Biochemical and histochemical findings confirmed the decreased proteins synthesis by mercury-intoxicated hepatocytes that were reflected by low serum albumin level and low protein content in hepatocytes concomitant with the ultrastructural change noticed in rough endoplasmic reticulum. This may be attributed to the formation of metal complex formed by interaction between mercury and intracellular thiol group of terminal cysteinyl residue of albumin molecule, and the formation of less stable complexes with other amino acid side chains which in turn can lead to enzymatic inactivation and inhibition of protein synthesis. Significant reduction in the serum total protein may be due to decline in protein synthesis by hepatic cells reflecting the hepatic dysfunction that accompanied by mercury treatment.
Mohamed et al., (2010), observed increase in total bilirubin and a decline of serum albumin level in the HgCl₂ administered group.

Administration of HgCl₂ showed elevated level of AST, ALT, serum ALP and GGT. Significantly (P < 0.05) with respect to control rats on the other hand NaMSA administered with HgCl₂ did not show such elevation and the values were closure to the control group significantly (P < 0.05). Morin treatments significantly decreased the elevated ALP levels by showing its antioxidant nature. These results are in agreement with earlier study where morin treatment reduced ALP levels against doxorubicin-induced toxicity in rats [38]. Morin hydrate has also been found to prevent necrosis of liver (Wu., 1993.; Wu., 1994).

The study was aimed to investigate the effect of different doses of morin-5'- sulfonic acid sodium salt on mercuric chloride induced biochemical changes. The levels of hepatic serum biochemical markers Aspartate transaminase, Alanine transaminase, Alkaline phosphatase, total bilirubin, Gamma glutamyl transferase, at the same time total protein, whereas simultaneous administration of mercuric chloride morin-5-sulfonic acid sodium salt did not show much deviation from their normal value. From the obtained results it is determined that morin-5'- sulfonic acid sodium salt 50mg/kg b.wt and 100 mg/kg b.wt was having almost equal effect against mercury toxicity. Hence, morin-5-sulfonic acid sodium salt 50 mg/kg b.wt is fixed as optimum dose and found to be safer against the hepatogenous toxic effect of morin-5'-sulfonic acid sodium salt.

Values are means ± S.D for six rats. Values not sharing a common superscript differ significantly at P<0.05 (DMRT)

**Figure 1: Effect of NaMSA on HgCl₂ influenced hepatic enzyme markers activity in the serum of control and experimental rats (IU/L)**

Mercuric chloride; NaMSA – Morin sulfonic acid -5'-sodium salt

Values are means ± S.D for six rats. Values not sharing a common superscript differ significantly at P<0.05 (DMRT)

**Figure 2: Effect of NaMSA on HgCl₂ influenced serum ggt activity in the control and experimental rats (IU/L)**

**Figure 3: Effect of NaMSA on HgCl₂ influenced serum total bilirubin level in the control and experimental rats**

**Figure 4: Effect of NaMSA on HgCl₂ induced plasma total protein level in the control and experimental rats (g/dl)**

Mercuric chloride; NaMSA – Morin sulfonic acid -5'-sodium salt

Values are means ± S.D for six rats.
Values not sharing a common superscript differ significantly at P < 0.05 (DMRT)

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