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Preliminary report

Effect of morin-5′-sulfonic acid sodium salt on the expression of apoptosis related proteins caspase 3, Bax and Bcl 2 due to the mercury induced oxidative stress in *albino* rats

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ABSTRACT

Many environmental contaminants have been reported to disturb the pro-oxidant or antioxidant balance of the cells by inducing oxidative stress. Oxidative stress mediated by the $HgCl_2$ induces DNA, protein and lipid oxidation resulted in necrosis or apoptosis, or both. Currently flavonoids are being emerging topic and reported to have antiviral, anti-inflammatory, anti- tumor and antioxidant activities. Morin is one of the flavonoid protects the cells from oxygen free radical damage and scavenges the free radicals and metals and also heals the injured cells commercially. Morin hydrate is sparingly soluble in water. Hence, the water soluble morin -5′- sulfonic acid sodium salt (NaMSA) was selected and synthesized. Aim of the present study was to analyze the effect of morin-5′-sulfonic acid sodium salt on the expression of apoptosis related proteins caspase 3, Bax and Bcl 2 due to the mercury induced oxidative stress in albino rats.. The experimental rats were exposed to sub lethal concentration of mercuric chloride (1.25 mg/kg) and the ameliorating effect of NaMSA was studied by using apoptotic protein markers Bax and caspase-3 and Bcl-2. The obtained results were analyzed using one way analysis of variance by the Duncan's Multiple comparison test to determine the level of significance (p) and $p < 0.05$ was considered as statistically significant. Administration of mercuric chloride (1.25 mg/kg) in the experimental rats increased the expression of Bax and caspase-3 and a decreased expression was noted in the Bcl-2 level compared with control bands significantly ($p < 0.05$). On the other hand NaMSA (50 mg/kg) and $HgCl_2$ (1.25 mg/kg) simultaneous administration did not bring any change in the protein expression of Bax, Caspase-3 and Bcl-2 levels compared with control rats. Hence, the membrane damage was protected, stopped the cell death and apoptosis. This could be due to the morin-5′-sulfonic acid sodium salt effective chelation action on the $HgCl_2$ generated free radicals.

1. Introduction

Mercury is a widespread environmental and industrial pollutant, induces severe alterations in the tissues of both animals and humans. It is still a worldwide problem ever since the outbreak of its poisoning in Minamata in Japan in the 1950s and in Iraq in 1971-72 [1]. About 100 tons of organomercurials are produced every year [2] from Fish, Pesticides in agriculture, fluorescent light bulbs, paints, cosmetics, air emissions, fungicide industry. It is a highly toxic metal results in a variety of adverse neurological, renal, respiratory, immune, dermatological, reproductive problems in target organs [3].

1.1. Mechanism

Multiple mechanisms proposed to explain the biological toxicity of HgCl 2 by investigating the biochemical fate of various forms of Hg [4]. Hg 2+ form has great affinity for

endogenous biomolecules-associated with thiol (-SH) group [5] and it is invariably attached to −SH-containing proteins, small-molecular weight peptides and amino acid [6], Consequently, the oxidative stress is one of the crucial mechanisms in Hg-induced pathological aspects [6] resulted in profound deterioration of vital metabolic processes [7,8].

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1.2. Oxidative stress and apoptosis

Mercury binds to proteins thiol groups [9] and decreased the glutathione level and increased the formation of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide and hydroxyl radicals [10], which provokes lipid, protein and DNA oxidation [11].

Apoptosis is programmed cell death, which is an important part of normal cell development and function of organisms. It can be triggered in a cell through either extrinsic pathway or the intrinsic pathway. The extrinsic pathway is initiated through the stimulation of the trans membrane death receptors, the intrinsic pathway is initiated through the release of signal factors by mitochondria within the cell. It is characterized by cell shrinkage, cytoplasmic, nuclear and chromatin condensation, membrane bleeding, protein fragmentation, DNA degradation and finally the breakdown of the cell into smaller units (apoptotic bodies) followed by (secondary) necrosis [12].

Oxidative stress is also an important mediator in the production of ROS which is associated with many forms of apoptosis [13]. Depending on its severity, lead to necrosis, apoptosis, or both [14].

Mercury induced ROS were involved in p38 activation. It has been reported that many proteins are involved in this complex process. Caspases, a family of cysteine dependent aspartate directed proteases, play critical roles in the initiation and execution of apoptosis [15]. Among the family of caspases, caspase-3, in particular, is believed to be the most commonly involved mechanisms in the execution of apoptosis in various cell types [16]. Whereas p38 mediated caspase activation regulates mercury-induced apoptosis [17], p38 mediated caspase activation regulated mercury- induced apoptosis [18]. Protein Bcl-2, a member of Bcl-2 family, is reported to inhibit apoptosis in a variety of cell types [19].

1.3. Therapeutic approaches

In the past 50 years considerable time and efforts have been made for mobilizing and removing the mercury from organs and tissues [20].

1.3.1. Chelation theraphy

The chelation process appears as an inevitable tug of war between the chelating agents and the competing biological ligands [21] and the same process is recommended by some by some physicians for treating mercuric poisoning with DMSA, 2, 3-dimercapto-1-physicians for treating mercuric poisoning with DMSA,2,3-dimercapto-1-propanesulfonic acid (DMPS), D-Penicillamine (DPCN), Dimercaprol, Glutathione and N-acetylcysteine (NAC), but they increased mercuric concentrations in the kidneys and the brain [22]. 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 nullify mercury poisoning. Currently flavonoids are being emerging topic which has more medicinal value.

1.3.2. Flavonoids

Over 4000 different flavonoids identified and they are most abundant in apples, red fruits, onions, citrus fruits, nuts and beverages such as tea, beer and wine

1.3.3. Morin (morin hydrate)

Synonyms: 2′, 3, 4′, 5, 7- Penta hydroxyl flavone; Molecular formula: C15H10O7. xH2O, Molecular Weight: 302.24 (anhydrous basis), Color- Natural yellow 11, Form: powder, Solubility-Methanol: soluble 50 mg/ml, Water: Sparingly soluble.

Morin hydrate is a flavonoid with antioxidant properties to protect the cells against oxygen radical damage *in vitro* [23], not only scavenges oxy radicals, but also moderately inhibits xanthine oxidase, a free-radical generating enzyme. Commercially available morin hydrate is sparingly soluble in water. The soluble sulfonated derivative of morin-5′ – sulfonic acid sodium salt was synthesized (NaMSA) [24,25]. Hence, the aim of the present work was to analyse the effect of morin-5′-sulfonic acid sodium salt on the expression of apoptosis related proteins caspase 3, Bax and Bcl 2 due to the mercury induced oxidative stress in albino rats.

2. Materials and methods

2.1. Chemicals used

The fine chemicals used for the present study purchased from Merck companies, Mumbai, India. Mercuric chloride, Morin hydrate and DNA Primers w e r e obtained from sigma Aldrich, USA. Antibodies obtained from Santa C ruz, USA and the rest of the chemicals and biochemical utilized were of analytical grade obtained from local firms (India).

2.2. Laboratory animals

Wister strain *albino* (male) rats with the age of 2 months weighing 180–220 g was used. The animals were housed in spacious cages under hygienic condition and maintained on commercial diet, 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. APCAS/IAEC/2010/01.

2.3. Preparation of morin-5′-sulfonic acid sodium salt (NaMSA) from morinhydrate

1 g Morin was added into 4 ml sulphuric acid in a 50 ml glass beaker placed on an ice bath and stirred for 2 h until complete sulfonation reaction was achieved. The mixture solution was then neutralized with 6 M sodium hydroxide, to which MeOH was added in order to dissolve the product at 40C. After stirring gently for 3 h, the solution was centrifuged at 3,000 x *g* for 15 min the precipitated fraction was collected. Methanol was evaporated in a rotary evaporator to obtain a yellow powder residue and then freeze-dried (Liu Wen *et al.,* 2012*;* Kopacz, 2003). The yield weight was 55 mg and water soluble. Molecular composition of the product was confirmed by elemental analysis of C, H, and S and also the structural characteristics determined by Fourier transform IR FT IR and in earlier papers [26–29].

2.4. Experimental design

A total number of 24 rats were used for the present study, and grouped them into

Group A: (Control): Rats orally administrated with 0.9% saline by stomach tube (at the volume of mercuric chloride/day) for 30 days.

Group B: Rats administered with mercuric chloride orally by stomach tube (1.25 mg/kg p.o.) dissolved in 0.9% saline for continuous 30 days. The dosage of HgCl2 was determined from the study performed by Elizabeth [30].

Group C: Rats administered with mercuric chloride orally by stomach tube (1.25 mg/kg p.o.) dissolved in 0.9% saline for continuous 30 days followed by morin-5′-sulfonic acid sodium salt orally (50 mg/kg p.o.) dissolved in water simultaneously for 30 days (Venkatesan and Mohamed Sadiq., 2013).