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Original article

Nephro-protective significance of kaempferol on mercuric chloride induced toxicity in Wistar albino rats

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ABSTRACT

The kidney is an imperative intention of the toxicity of drugs, xenobiotics, and oxidative stress. The intend of the present investigation was to conclude nephro-protective efficiency over mercuric chloride ($MgCl_2$) induced oxidative stress and renal injury in rats. Mercuric chloride induced nephrotoxicity (1 mg kg bw; i.p.) as it induces noticeable and characteristic changes in proximal tubular cells in group II animals. These changes are conduit and accompanied by signs of renal dysfunctions. The levels of blood urea, serum creatinine, uric acid, and lipid peroxidation were elevated ($P < 0.05$) in heavy metal intoxicated group II animals. On the other hand there was a decline in the levels of serum protein, nucleic acids, enzymic and non-enzymic antioxidant enzymes in group II toxicity induced rats. In group III, rats administration of kaempferol (100 mg kg bw p.o.) brought back these elevated urinary constituents, lipid peroxidation ($P < 0.05$) and increase the levels of antioxidants and nucleic acids levels to near normal ($P < 0.05$) due to its therapeutic and scavenging properties. Histopathological results muscularly supports the nephro-protective activities of kaempferol by convalescing oxidative damage and morphological changes of kidneys induced by $MgCl_2$. Therefore, the present analysis evidently showed that kaempferol may be useful due to antioxidant possessions in fighting against free radical-induced oxidative stress and tissue injury resulted from mercuric chloride induced nephrotoxicity.

1. Introduction

Humans are exposed intentionally and unintentionally to a range of diverse chemicals that damage the kidney. The increasing use of therapeutic drugs, natural products, environmental pollutants and industrial chemicals for the last few decades has considerably increased the possibility of damage to the kidney and it has become clear that chemicals with diverse chemical structures produce nephrotoxicity [1,2]. Heavy metals are known to induce cell injury in the kidney and may be major contribution to nephrotoxicity [3,4]. Mercury is environmental pollutants and effective toxic metal with significant health effects in humans, including nephrotoxicity that are released into the environment mainly through anthropogenic action [5,6]. Various species of mercury, which includes charged inorganic mercurous (Hg^{1+}), mercuric salts (Hg^{2+}), neutral elemental metal (Hg^0) and organic molecules in which human are exposed. The indication of nephrotoxicity caused by mercury in man and experimental animals reflect reabsorptive and secretary defects largely concentrated in peroximal tubules [7].

Mercuric chloride ($HgCl_2$) affects the oxidative function because of its high affinity for cellular cysteine thiols [8]. $HgCl_2$-induced damage is strictly dependent on the route of administration, time and dose [9]. Exposure of animals to mercuric compounds induces an oxidative stress, which was monitored by the accumulation of lipid peroxidation products [10], production of reactive oxygen species [11] and decreases in antioxidant enzymes [12], reduced ATP content [13]. Inorganic mercury ($HgCl_2$) has been shown to accumulate in kidneys [14] along with in other organs. Mercuric chloride administration is a classic model for the study of the pathogenesis of inorganic mercury toxicity in both *in vitro* and *in vivo* systems [15]. A specific concern associated with mercury exposure in humans is the need for effective therapy in dealing with intoxication.

Natural dietary agents play a major role in prevention of various free radical-induced oxidative stress [16]. Flavonoids are natural polyphenolic products that are ubiquitous in vegetables and fruits, which are potent antioxidants and components in human diet

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[17,18]. Kaempferol (3,4′,5,7-tetrahydroxyflavone) have received a lot of attention due to their anti-carcinogenic, cardioprotective and anti-aging properties [19,20]. These properties have been attributed to their ability to act as antioxidants, anti-inflammatory agents, and pro-apoptotic agents. Kaempferol have also been reported to inhibit the enzymatic activities of some phases I and II drug metabolizing enzymes [21,22]. The present study has been designed to investigate the influence of kaempferol on $MgCl_2$ intoxicated kidney tissue of experimental rats.

2. Materials and methods

2.1. Chemicals

Mercuric chloride ($HgCl_2$) was purchased from Hi-Media laboratories Ltd. Mumbai, India. Kaempferol were purchased from Sigma Saint-Louis, MO, USA. All other chemicals including solvents used were of high purity and of analytical grade.

2.2. Experimental animals

Healthy male Wistar albino rats of weighing between 120 ± 10 g were used for the study. They were obtained from the Central Animal House Facility, Dr. Almpgibms, University of Madras, Chennai (IAEC No: 06/02/11). The animals were kept in polypropylene cages and received standardized rat pellet and water *ad libitum*. All the procedures were done in compliance with the guidelines issued by the Institutional Animal Ethics Committee.

2.3. Experimental protocol

The experimental animals were divided into four groups of six animals each. Group I control animals were administered orally with 1 mL of DMSO. To induce nephrotoxicity, Group II animals were intoxicated with $MgCl_2$ (1 mg kg bw; i.p.) weekly thrice for 1 month. After 45 days, Group III nephrotoxicity induced rats were treated with kaempferol (100 mg kg bw p.o.) dissolved in 1 mL of DMSO for a period of 28 successive days. Group IV animals received kaempferol alone (100 mg kg bw p.o.) dissolved in 1 mL of DMSO for a period of 28 successive days.

2.4. Collection of samples

At the end of the experimental period, all the animals were sacrificed by cervical decapitation. The urine collected on ice was free from faecal contamination. Urine samples were centrifuged and aliquots separated. One portion was acidified with concentrated HCl and used for analysis of urea, uric acid and serum creatinine. The remaining was dialyzed at 4 °C against distilled water for 3 hours and later was used for the determination of various enzymes and proteins.

2.5. Collection of tissue sample

At the end of the experiment, all the animals were sacrificed by cervical decapitation. Blood was collected in tubes containing EDTA and centrifuged at 3,000 rpm for 15 minutes. The buffy coat was removed and the packed cells were washed thrice with physiological saline. The washed cells were lysed by suspending in hypotonic buffer and centrifuged at 15,000 g for 30 minutes. The resulting pellet is the erythrocyte membrane and the supernatant represent the hemolysate. Kidneys were perfused *in situ* with cold 0.15 M NaCl at 37 °C for enzyme assays.

2.6. Biochemical analysis

Estimation of blood urea [23], serum creatinine [24], uric acid [25] and protein [26]. Estimation of DNA [27] and RNA [28], assay of lipid peroxidation [29], peroxide induced lipid peroxidation, ascorbate induced lipid peroxidation and ferrous induced lipid peroxidation [30]. Estimation of antioxidant enzymes such as superoxide dismutase [31], catalase [32], glutathione peroxidase [33], glutathione [34], ascorbic acid [35] and α-Tocopherol [36].

2.7. Tissue processing for histopathological studies

After sacrifice, the animals were autopsied. Their kidney were rapidly excised and placed in 10% formalin for 24 hours to fix the tissues. They were then washed in running tap water and dehydrated by using increasing concentrations of isopropanol and fixed in xylene. The tissues were then blocked in molten paraffin wax and serially sectioned with a razor blade. The sections were stained with hematoxylin and eosin (H and E).

3. Results

The levels of urinary constituents are shown in Fig. 1. The levels of blood urea, serum creatinine and uric acid were increased and protein a level was decreased in group II nephrotoxicity induced rats when compared with normal untreated control rats. On administration of natural bioflavonoid kaempferol, brought back these urinary constituents levels to near normal ($P < 0.05$) when compared with group II metal intoxicated rats. No noticeable changes in these urinary constituents in group IV drug control rats when compared with untreated control group I animals.

Fig. 2 illustrates the levels of nucleic acids in the kidney of control and experimental animals. The nucleic acids such as DNA and RNA levels were significantly decreased ($P < 0.05$) in the toxicity induced group II rats when compared with group I rats. On kaempferol administration reverted back these DNA and RNA levels in group III animals ($P < 0.05$) when compared with group II rats. No changes were observed in nucleic acids levels in group IV when compared with group I control rats.

The effect of kaempferol on lipid peroxidation in the kidney of control and experimental animals represented in Fig. 3. The levels of basal, H_2O_2, ascorbate and $FeSO_4$ induced lipid peroxidation were significantly increased ($P < 0.05$) in $MgCl_2$ intoxicated group II animals when compared with group I animals. Fascinatingly, the levels of elevated LPO were statistical declined ($P < 0.05$) in group III animals due to treatment of bioflavonoid kaempferol when

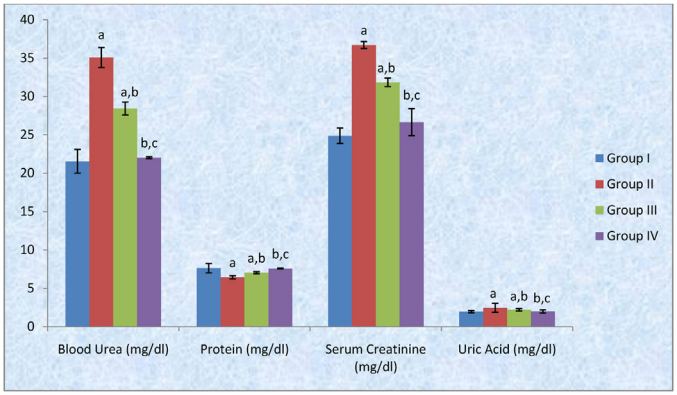

Fig. 1. Effect of kaempferol in urinary constituents of control and treated rats. Values are expressed as mean ± SD for six animals in each group: a: group I vs. group II, III and IV; b: group II vs. group III and IV; c: group III vs. group IV. The significance at the level of $P < 0.05$.