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ANTIOXIDANT ENZYME ACTIVITIES, LIPID HYDROPEROXIDE IN HUMAN ERYTHROCYTES MEDIATED BY MONO AND DI-CATION: POTENTIAL ROLE OF GALLIC ACID AND ROSEMARY EXTRACT

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ABSTRACT

The effect of Li^{+1}, Cs^{+1}, Ni^{+2}, Mo^{+2}, Mg^{+2} and Ba^{+2} ions on human erythrocyte antioxidant enzymes was studied. Three doses of each metal (10, 50 and 100 μM) were evaluated by their effect on the activity of catalase (CAT), glutathione peroxidase (GpX), glutathione reductase (GR) and glutathione S-transferase (GST) and lipid peroxide level (lipid peroxidation) indicated by malondialdehyde (MDA) formation. Also, the antioxidant activities of rosemary extract and gallic acid in the presence of these metals were examined. Compared to the control, GpX, CAT and GR activities were significantly increased, while the GST activity was significantly decreased in Ni^{+2} and Li^{+1} treated cells, whereas, Cs^{+1} caused a significant increase in GpX, CAT, and GST and decrease in GR activities. However, Ba^{+2} showed no significant differences in GpX activity, while CAT and GSTs increased and GR decreased significantly. Also, Mg^{+2} significantly increased the GpX, GR, CAT and GSTs activities. Mo^{+2} showed a significant decrease in GR activity at high concentration, while GpX, CAT and GSTs increased significantly. Li^{+1}, Cs^{+1}, Ni^{+2}, Mo^{+2} and Ba^{+2} caused an increase in MDA formation, whereas, Mg^{+2} at 100 μM caused a significant decrease in MDA compared to low concentrations. Gallic acid and rosemary extract inhibited lipid peroxidation (MDA) by 28 to 64% and 9 to 48%, respectively. The results of this study suggest that reactive oxygen species (ROS) might be involved in the Li^{+1}, Cs^{+1}, Ni^{+2}, Mo^{+2}, Mg^{+2} and Ba^{+2} toxicity and their ability to increase or maintain GpX, CAT, GST and GR activities and MDA formation. Gallic acid and rosemary extract as antioxidant were found to inhibit MDA production under oxidative stress conditions. Furthermore, these results indicate that gallic acid and rosemary are capable of protecting erythrocytes against ROS.

Key words: *antioxidant enzyme, blood oxidative stress, erythrocyte, heavy metals, malondialdehyde, rosemary.*

1. INTRODUCTION

Metal pollution is the focus of biological research because of their widespread use. The harmful exposure to heavy metals, either by occupation or environment is of biological and toxicological concerns. These metals lead to toxicity and may also mediate development of pathological condition in exposed individuals (DeRosa and Johnson, 1996).

Transition metals as a redox active elements, appear to play an important role in the etiology of cell damage as a result of exposure to metals and to metals-catalyzed reactive oxygen species (ROS) (Halliwell and Gutteridge, 1984; Sies, 1993; and Kasprzak, 2002).

Erythrocytes are the main target for the metal toxicities (Battistini *et al.*,1971), which lead to oxidative damage induced by ROS such as superoxide anion ($O_2^{\cdot}$) and hydroxy radicals ($OH^{\cdot}$) and non-free radical species such as hydrogen peroxide (H_2O_2) (Fridovich, 1997). ROS will initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxide and cellular injury. Lipid peroxidation as one of the indicators of oxidative stress in cells was determined by malondialdehyde (MDA) formation (Clemens and Waller, 1987).

Cells have developed different means to overcome the ROS toxicity which included non-enzymatic antioxidants, provided by food constituents (ascorbic acid, glutathione, tocopherols), enzymes regenerating the reduced forms of antioxidants and ROS–scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxides (GPx) and glutathione reductase (GR) (Ribarov *et al.*,1981;

Clemens and Waller, 1987; Frankel *et al.*,1996). ROS–scavenging mechanism includes: the dismutation of $O_2^{\cdot-}$ to H_2O_2 catalysed by SOD, and the conversion of H_2O_2 to H_2O by GpX or CAT and glutathione regeneration by GR (Pigeolet *et al.*,1990; Sies, 1993 and Michiels *et al.*,1994).

Plants, including herbs and spices are potential sources of natural antioxidants such as tocopherols, flavonoids, tannins and phenolic acids. The greatest level of attention among herbs and spices as sources of antioxidants has been focused on rosemary (*Rosmarinus officinalis* L), which is widely used in many food applications, especially in Mediterranean dishes (Cuvelier *et al.*,1996; Frankel *et al.*,1996; Martinez-Tome *et al.*,2001; Moreno *et al.*,2006; Erkan *et al.*,2008; and Hernández-Hernández *et al.*,2009).

The effects of mono and dication metals on erythrocytes antioxidant system still poorly understood either *in vitro* or *in vivo*. Therefore, the purposes of the present work were to determine the potential changes in antioxidants enzymes activities such as CAT, GpX, GR and GST; lipid peroxidation (MDA) levels, when the erythrocytes are exposed to metals (Li^{+1}, Cs^{+1}, Ni^{+2}, Mo^{-2}, Mg^{+2} and Ba^{+2}) ions *in vitro*. Also, to investigate the inhibition of MDA formation by antioxidants such as gallic acid as pure compound and rosemary extract as complex plant matrices.

2. MATERIALS AND METHODS

2.1. Chemicals

Glutathione reductase, t-butylhydroperoxide, reduced glutathione, gallic acid and 1-chloro-2,4-dintrobenzene were purchased from Sigma - Aldrich Chemical Co. (Germany). Magnesium chloride was obtained from Fluke (Germany). Hydrogen peroxide and cesium chloride were from GCC (England). Barium chloride was purchased from BDH England. NADPH and oxidized glutathione were purchased from Applichem (Germany). Lithium chloride was purchased from Pharmacos ltd (England). Nickel chloride $6H_2O$ was purchased from Philip Harris England. Sodium molybdate, was obtained from SDS Fine Chemical Company (India). Hemoglobin Reagent Kit from Techo Diagnostics, USA.

2.2. Rosemary extract preparation

Leaves of *Rosmarinus officinalis* L. were collected from Mutah University campus area (Southern of Jordan) in May 2008 and dried at room temperature (25 °C) for 3 days. Then chopped and extracted with 80% ethanol for 12 h at room temperature. The extract was then filtered, evaporated under reduced pressure to obtain a viscous mass of dark green extract. An aliquot of the extract was solubilized in ethanol to a final concentration of 1.0 mg/mL.

2.3. Preparation of blood sample

Blood was collected into test tubes containing ethylene diaminetetra acetate (EDTA) from normal human volunteers and not occupationally exposed to heavy metals. EDTA–blood was centrifuged; the clear plasma and puffy coat layers were discarded. The RBC suspension was filtered through cotton wool to remove any leftover leukocytes. RBC were washed with cold 0.15 M sodium chloride solution three times after a 1- to-10 dilution. The hemolysates were prepared then by adding 0.9 ml of distilled water to 0.1 ml of diluted RBC, and they were frozen at -20 °C for future analysis. Hemoglobin concentration was determined by hemoglobin kit.

2.4. *In Vitro* metals treatment

Phosphate-buffer (pH 7.4) was used to dilute the washed erythrocytes (1 : 10). Aliquots of the cell suspension were placed in 15 ml screw caped test tubes in the presence of 10, 50 and 100 μM of cesium chloride (Cs^{+1}), lithium chloride (Li^{+1}), magnesium chloride (Mg^{+2}), barium chloride (Ba^{+2}), nickel chloride (Ni^{+2}) and sodium molybdate (Mo^{-2}). The contents of the tubes were incubated win the shaking in water bath at 37°C for 12 h. 0.2 mM EDTA- phosphate buffer was added to treated erythrocytes before biochemical analyses. The control without metals was included each time.

2.5. Hemoglobin measurement

The hemoglobin concentration was determined by hemoglobin Reagent Kit from Techo Diagnostics, USA.

2.6. Antioxidants enzymes assay

The activities of GST, GR, GpX and CAT were determined spectrophotometrically in red blood cells hemolysates after incubation with metals for 12 h. GSTs activity was measured by the spectrophotometric assay of Habig *et al.* (1974). GR activity was assayed as described by Gupta *et al.*(1999). GpX activity with t-butyl-hydroperoxide was determined according to the method of Lawrence and Burk (1976). CAT activity was measured spectrophotometrically by monitoring the decrease in A_{240} resulting from the elimination of H_2O_2 as described by Aebi (1984). The specific activity of GSTs, GR, GpX and CAT was expressed as μmol/min/g hemoglobin (U/g Hb). Blank reactions with lytic cell replaced by distilled water were subtracted from each assay.

2.7. Lipid peroxide assay

The MDA levels were measured by the double heating method (Draper and Hadley, 1990). The erythrocytes were lysed with distilled water and then used for MDA determination. The principle of the method was based on the spectrophotometric measurement of the color developed during the reaction of thiobarbituric acid (TBA) with MDA. The concentration of TBA-MDA complex was calculated (absorbance coefficient E = 1.56 × 105 cm–1 M–1 at 532 nm) and expressed as μmol/g Hb.

2.8. Gallic acid and rosemary extract: anti-lipid peroxidation

An erythrocyte suspension (10%) was incubated with PBS (control) and preincubated with rosemary extracts (100 μg/mL) or gallic acid (100 μg/mL) separately at 37 ^{o}C for 30 min, followed by incubation with and without metals (100 μM) in PBS at pH 7.4. This reaction mixture was shaken gently while being incubated for a 12 h at 37 ^{o}C. One ml of 1:1 ratio of the reaction mixture and a hypotonic buffer (5 mM phosphate buffer at pH 7.4; 100% hemolysis) was removed and used for the MDA analysis.

2.9. Statistical analysis

Data are expressed as means ± SE (n=5) (results obtained from 5 different experiments). Significant differences between the control and heavy metal treated red cells were determined by ANOVA. Differences were considered significant when $P<0.05$.

3. RESULTS

3.1. GST activity

The GST activity was increased significantly ($p<0.05$) when exposed to 10 μM of Cs^{+1}, Mg^{+2} and Mo^{+2}. Whereas, the Ni^{+2} caused a significant decrease in GST activity relative to the control. Moreover, at high concentration up to 100 μM Ba^{+2}, a significant change in GST activity was detected. Also, Cs^{+1}, Li^{+1}, Ni^{+2} and Mo^{-2} at 100 μM caused a significant decrease in GST activity (Table 1).

3.2. GpX activity

The GpX activity showed a statistical significance changes ($p<0.05$) when the erythrocytes incubated with 10 μM Cs^{+1}, Li^{+1}, Ni^{+2}, Ba^{+2}, Mg^{+2} and Mo^{+2} and their activity was increased by 44.3%, 28.8%, 18%, 12.4%, 51.2%, and 32.4%, respectively. Moreover, at high concentration up to 100 μM of Mg^{+2}, no significant change in activity was detected. However, GpX displayed no changes in activity toward Cs^{+1}, Li^{+1}, Ni^{+2}, Ba^{+2} and Mo^{-2} at 100 μM when compared to the effect at low concentrations (Table 1).

3.3. CAT activity

In Table 1, CAT activity was increased (35%) at 50 μM Mo^{+2} and no further increase at 100 μM Mo^{+2}, was detected. Also, CAT activity increased significantly ($p<0.05$) up to 138% by increasing the Mg^{+2} concentration.. The CAT showed 2 fold increments in activity at 100 μM Mg^{+2}. Also, Ba^{+2} caused a significant increase in erythrocytes CAT activity ($p<0.05$) and it was concentration dependent when compared to the control (Table 1).

3.4. GR activity

An overall no significant changes in GR activity occurred in treated cells at 10 μM in comparing to the control. However, it was significantly increased by 314% and 52% at 100 μM Li^{+1} and Ni^{+2}, respectively ($p<0.05$). Moreover, the GR activity was significantly inhibited at 100 μM Cs^{+1}, Ba^{+2} and Mo^{+2} by 54%, 59 % and 59% respectively when compared to the control (Table 1).

3.5. Lipid peroxide (LPO)

A significant increase in the level of LPO, measured as MDA content, was observed in all treated erythrocytes at all concentrations, being maximum with erythrocytes + 100 μM Ni^{+2} (191%) followed by Cs^{+1} (89%), Ba^{+2} (79%), Li^{+1} (71%), Mo^{-2}(64%), and Mg^{+2} (52%) after 12 h. Moreover, the MDA level was increased significantly as the metal concentration increased except in case of Li^{+1} and Cs^{+1} decreased significantly when compared to normal. Also, Li^{+1} and Cs^{+1} increased the MDA level at 10 μM more than the other metals. The oxidizing power was Li^{+1}> Cs^{+1}> Mg^{+2}> Ba^{+2}, Mo^{-2}>Ni^{+1} and the MDA was 158, 63, 44, 40,32 and 23%, respectively (Table 2).

3.6. Anti-lipid peroxidation of gallic acid and rosemary extract

The MDA formation induced by the metals after rosemary extract and gallic acid treatment is summarized in (Table 3). The rosemary extract and gallic acid decreased MDA levels significantly, in erythrocytes treated with and without metals when compared to the control. On the other hand, gallic acid showed more pronounced antioxidant activity against lipid peroxidation. A decrease in the level of MDA was observed in Cs^{+1}, Li^{+1}, Ni^{+2}, Mo^{-2}, $Mg^{+2}$and Ba^{+2} treated cells at 100 μM + 100 μg/ml gallic acid, being maximum with gallic acid + Ni^{+2} (64 %), Li

Table (1): Effect of some mono and di-cations on human erythrocytes antioxidant enzyme activities after 12 h incubation.

Parameters / Metals	Human erythrocytes antioxidant enzymes			
	CAT (U/g Hb)	GpX (U/g Hb)	GR (U/g Hb)	GST (U/g Hb)
Control	4.18^{a}	11.05^{a}	1.24^{a}	3.92^{a}
Cs^{+1}				
10 µM	7.06^{b}	15.94^{b}	0.92^{b}	4.82^{b}
50 µM	6.25^{b}	21.54^{c}	0.70^{bc}	4.60^{b}
100 µM	11.35^{c}	13.00^{a}	0.57^{c}	4.02^{a}
SE	1.36	1.86	0.26	0.11
Li^{+1}				
10 µM	5.39^{b}	14.23^{b}	1.40^{a}	4.15^{a}
50 µM	5.55^{b}	12.99^{a}	1.78^{a}	3.93^{a}
100 µM	3.92^{a}	12.32^{a}	5.13^{b}	3.65^{b}
SE	0.57	1.23	0.37	0.1
Ni^{+2}				
10 µM	6.10^{b}	13.05^{b}	0.70^{a}	3.34^{b}
50 µM	4.91^{a}	13.09^{b}	1.64^{b}	3.44^{b}
100 µM	4.72^{a}	13.25^{b}	1.88^{b}	3.54^{b}
SE	0.65	0.51	0.37	0.15
Ba^{+2}				
10 µM	5.25^{a}	13.67^{b}	1.55^{a}	4.23^{a}
50 µM	6.67^{b}	11.79^{a}	1.25^{a}	4.67^{b}
100 µM	6.83^{b}	12.02^{a}	0.51^{a}	4.95^{b}
SE	1.14	1.25	0.26	0.42
Mg^{-2}				
10 µM	5.03^{b}	16.71^{b}	1.37^{a}	4.34^{b}
50 µM	5.73^{b}	13.93^{c}	1.88^{b}	4.32^{b}
100 µM SE	9.98^{c}	9.95^{d}	1.22^{a}	4.32^{b}
	0.49	0.47	0.36	0.1
Mo^{-2}				
10 µM	4.90^{a}	14.63^{b}	1.47^{a}	4.67^{b}
50 µM	5.64^{b}	14.48^{b}	1.56^{a}	4.34^{b}
100 µM	5.13^{a}	14.6^{b}	0.51^{b}	3.76^{a}
SE	0.35	0.31	0.27	0.15

Enzyme activities are represented by mean values of five experiments (U/g Hb). a,b,c,d: Different superscripts show significant differences in the same column ($P < 0.05$). CAT: Catalase; GpX: glutathione peroxidase; GR: glutathione reductase ;GST: glutathione transferases. SEM.: mean standard error

Table (2): Malondialdehyde concentration after 12 h incubation of human erythrocytes in solutions of metal salts.

Metal	Erythrocytes MDA (μM/g Hb)				SE	Sig.
	Without	with 10 μM	with 50 μM	with 100 μM		
Cs^{+1}	0.98 [a]	1.60 [b]	1.57 [b]	1.85 [c]	0.06	***
Li^{+1}	0.98 [a]	2.53 [b]	1.78 [c]	1.68 [c]	0.16	*
Ni^{+2}	0.98 [a]	1.21 [b]	1.88 [c]	2.85 [d]	0.08	**
Ba^{+2}	0.98 [a]	1.37 [b]	1.48 [b]	1.75 [c]	0.04	**
Mg^{+2}	0.98 [a]	1.42 [b]	1.92 [c]	1.49 [b]	0.13	*
Mo^{+2}	0.98 [a]	1.30 [b]	1.50 [c]	1.61 [c]	0.08	**

MDA concentrations are represented by mean values of five experiments and expressed as (μmole/ g Hb).
*, **,***ANOVA tests among concentrations (in the same line) at $P < 0.05$, $P < 0.01$ and $P < 0.001$ significantly different from the control, respectively. [a,b,c,d] Different superscripts show significant differences between the concentrations in the same line. SEM: mean standard error.

Table (3): The effect of Rosemary extract and gallic acid on the level of MDA after 12 h incubation of human erythrocytes with metal salts.

Metal	Erythrocytes MDA (μM/g Hb)				
	Phosphate buffer	Rosemary extract	Gallic acid	SE	Sig.
Without	0.98 [a]	1.03 [a] (+5%) [&]	0.45 [b] (-54%)	0.02	***
With Cs^{+1}	1.85 [a#]	1.69 [a] (-9%)	1.08 [b] (-42%)	0.06	**
Li^{+1}	1.68 [a#]	1.38 [b] (-18%)	0.62 [c] (-63%)	0.05	**
Ni^{+2}	2.85 [a#]	1.78 [b] (-38%)	1.04 [c] (-64%)	0.07	**
Ba^{+2}	1.75 [a#]	0.91 [b] (-48%)	1.26 [c] (-28%)	0.11	*
Mg^{-2}	1.49 [a#]	1.26 [b] (-15%)	0.64 [c] (-57%)	0.08	**
Mo^{-2}	1.61 [a#]	1.01 [b] (-37%)	0.75 [c] (-53%)	0.05	***

MDA concentrations are represented by mean values of five experiments and expressed as (μmole/ g Hb).
*, **,***ANOVA tests among concentrations (in the same line) at $P < 0.05$, $P < 0.01$ and $P < 0.001$ significantly different from the control, respectively.
[a,b,c.] Different superscripts show significant differences between the rosemary, gallic acid and metal in the same line.
Sig. difference between the control and metal in the same column. [&](MDA inhibition or activation %).

$^{+1}$ (63 %), followed by Mg^{+2} (57 %), Mo^{+2} (53 %), Cs^{+1} (42 %) and Ba^{+2} (28%) after 12 h. While a significant decrease in MDA level ranged from 9 to 48 % was observed in the human erythrocytes treated with rosemary extract.

4. DISCUSSION

Human erythrocytes represent a useful model to evaluate the effects of ROS. The erythrocyte has a high susceptibility to oxidative stress, because it contains a large amount of polyunsaturated fatty acids. It is also, exposed more to oxygen, which leads to lipid peroxidation. This plays an important role in the toxicity of many xenobiotics (Oostenbrug *et al.*,1997; Anane and Creppy, 2001). The erythrocyte has a physiological importance as well as antioxidant capacity (Mendiratta *et al.*, 1998 and Siems *et al.*, 2000). It comprises an intracellular antioxidant enzymes such as SOD, CAT, GpX, GR and GSTs. The present study reports the effects of mono- and di-cations (*i.e.*, Cs^{+1}, Li^{+1}, Ni^{+2}, Ba^{+2}, Mg^{+2}, Mo^{+2}) on the lipid peroxidation, and the antioxidant systems in human erythrocytes *in vitro*.

Oxidative damage as a result of ROS, leads to the formation of metHb, which will be reduced to ferrohemoglobin through several antioxidants to restore hemoglobin function. The reduction of metHB and GSH is achieved by the action of NADH and NADPH, derived from the glycolysis and oxidative pentose phosphate pathways. GSH in erythrocytes acts in the antioxidant GSH defense system, particularly metHb reductase, GpX, GR,GST and CAT, as well as the small molecules ascorbate and vitamin E (Siems *et al.*,2000). In the absence of an efficient antioxidant systems, oxidative stress will cause the release of an active form of iron (Fe^{+3}). If the oxidative stress continues, sequential formation of harmful molecules occurs, leading to oxidative damage due to chronic redox imbalances which cause the hemolytic in the erythrocytes, because of membrane damage, *via* the Fenton -type reaction (Jain *et al.*,1983; Pardo-Andreu *et al.*, 2005).

The obtained results showed that Cs^{+1}, Li^{+1}, Ni^{+2}, Ba^{+2}, Mg^{+2} and Mo^{+2} have a role in the induction of oxidative stress indicated by a significant enhancement of MDA production, in the treated erythrocytes. This increase may be associated with the metal activities as redox molecules. This is in accordance with previous works of Prakash and Rao (1995), Anane and Creppy (2001), Dua and Gill (2001) and Al-Mustafa (2006).

A previous work shows that ROS formation in the erythrocytes will be formed because of the interaction between hemoglobin and redox metals (Winterbourn, 1983). This oxidative stress gives rise to the $O_2^{\cdot-}$, H_2O_2 and peroxy radicals. This will induce membrane lipid peroxidation and hemolysis (Clemens and Waller, 1987). The generation of ROS may be also a result of interaction between metal and erythrocyte membranes. It was found that the interaction of $CuCl_2$ with erythrocyte membrane is accompanied with $O_2^{\cdot}$ and H_2O_2 generation. Therefore, it initiates peroxidative processes in erythrocytes, and ends with the hemolysis (Stocks and Dormandy, 1971; Ribarov and Bochev, 1984).

The erythrocyte antioxidant enzymes contribute to the maintenance of a relatively low level of the ROS generated through the Haber–Weiss reaction and Fenton reaction (Hidalgo *et al.*, 2002; Sanchez *et al.*, 2005). When erythrocytes were treated with Cs^{+1}, Li^{+1}, Ni^{+2}, Ba^{+2}, Mg^{+2} and Mo^{+2} *in vitro*, the activities of GSTs, GR, CAT and GpX were altered (Table 1).

GSTs play an important role in detoxification and metabolism of the lipid hydroperoxides, many xenobiotic and endobiotic compounds. The decrease in the activity of GSTs in human erythrocytes treated with metals was found in the studies of Hunaiti and Soud (2000) and Al-Mustafa (2006). This might be a result of a decrease of GSH level, because the GST consume the GSH during the peroxide and metals detoxification (Hunaiti and Soud, 2000). The metals alter the activity of antioxidant system such as GST, CAT, GpX and GR, because of ROS production, through intracellular thiols oxidation (*i.e.*, GSH) and /or via interaction with thiols which are central components of redox-sensitive proteins (Kasprzak, 2002). Also, GSH play a role as intracellular cations chelator through the cysteine residue (Aust *et al.*,1985), in addition to serving as a substrate for the GpX, GR and GSTs. Its regeneration is also affected by metals, therefore, it affects the GSH dependent enzyme activities (Grose *et al.*,1987; Ochi *et al.*,1988; and Mates, 2000).

The GpX activity was increased after treatment with Cs^{+1}, Li^{+1}, Ni^{+2}, Ba^{+2}, Mg^{+2} and Mo^{+2}. This is due to $O_2^{\cdot}$, H_2O_2 and lipid peroxide formation. GpX, is responsible for enzymatic defense against H_2O_2 and is strictly dependent on the concentration of GSH because it catalyses the reaction between GSH and H_2O_2, resulting in the formation of glutathione disulphide (GSSG). Furthermore, GpX activity is linked to the activity

of GR, which supplies reducing equivalent for GpX function to maintain the cell integrity and its antioxidant system (Paglia and Valentine, 1967).

The present results also showed that CAT activity was significantly increased with gradual increase of Cs^{+1}, Ba^{+2} and Mg^{+2} concentrations ($p<0.001$), whereas, dual effects were found on Li^{+1} and Ni^{+2}- treated cells. Moreover, CAT inhibition previously, related to the accompanied direct binding of metal ions to –SH groups on the enzyme molecule, caused the increase in O_2^-, H_2O_2 due to oxidative stress. It was indicated that rapid inactivation of CAT at high hydrogen peroxide concentration was due to the converting of active enzyme to inactive compounds (Wong and Whitaker, 2002).

GR was significantly inhibited at 100 μM Cs^{+1}, Ba^{+2} and Mo^{+2}. Such inhibition has previously observed in carp tissues treated with Cu^{+2} (Winston and Di Giulio, 1991) and in sea bass erythrocytes (Roche and Boge, 1993). This may be due to the inhibition of pentose phosphate pathway, which is important for the regeneration of GSH.

Many studies have been performed on the properties of Rosemary (*Rosmarinus officinalis* L.) in different systems and shown to be a potent scavenger of a variety of ROS and has been widely used as an antioxidant. Phenolic compounds, such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol have been identified as major constituents that contribute to the antioxidant activity of rosemary (Aruoma *et al.*,1992; Erkan *et al.*, 2008). Many reports indicated that rosemary extracts can retard lipid oxidation and prolong the shelf life of meat products through the break free radical chain reactions by donation hydrogen atoms. The ROS-scavenging and transition metal-chelating activities of Rosemary extract might contribute to the inhibitory effects on lipid peroxidation. Frankel *et al.* (1996) showed that rosmarinic acid reduces transition metals including iron and copper. In addition, rosmarinic acid can reduce Mo(VI) to Mo(V), preventing the product of free radicals caused by the metal (Petersen and Simmonds, 2003).

The data shown in (Table 3) reveal that gallic acid and rosemary extract has a marked capacity for lipid peroxidation inhibition, suggesting that its main action as a peroxidation inhibitor and may be related to its metals binding capacity. This provides an important antioxidative effects by retarding metal-catalysed oxidation (Halliwell and Gutteridge, 1990). Curcumin, tocopherol and trolox showed ferrous ion chelating activities (Dinis *et al.*,1994). Also, Kazazic *et al.* (2006) demonstrated that flavonoids, such as kaempferol, chelated Cu^{2+} and Fe^{2+} through the functional carbonyl groups .

In conclusion, these findings demonstrate that *in vitro* administration of Cs^{+1}, Li^{+1}, Ni^{+2}, Ba^{+2}, Mg^{+2} and Mo^{+2} to erythrocyte cells results in the induction of MDA and changes in antioxidant system, supporting that ROS may be involved in the toxic effects of these metals. While gallic acid and Rosemary extracts are capable of protecting erythrocytes against oxidative damage by reducing the MDA probably by acting as a strong antioxidant. Finally it is suggested that the *in vivo* effects of Cs^{+1}, Li^{+1}, Ni^{+2}, Ba^{+2}, Mg^{+2} and Mo^{+2} should be investigated further.

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نشاط إنزيمات مضادة الأكسدة والدهن المؤكسد في كريات الدم الحمراء للإنسان بواسطة العناصر موجبة الاستقطاب: دور حامض الجاليك ومستخلص نبات إكليل الجبل

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ملخص

تهدف هذه الدراسة الى معرفة تأثير بعض العناصر (Li^{+1}, Cs^{+1}, Ni^{+2}, Mo^{-2}, Mg^{+2}, Ba^{+2}) على نشاط الإنزيمات المضادة للأكسدة في كريات الدم الحمراء للإنسان (كتاليز CAT ، جلوتاثيون بيرأكسيديز GpX ، جلوتاثيون ريدكتيز GR ، وجلوتاثيون الناقل الكبريتي GST) ومستوى مالون ثنائي الألديهايد MDA و كذلك تأثير مستخلص نبات إكليل الجبل وحامض الجاليك على سمية هذه العناصر و تأثيرها على الــ MDA .

أوضحت النتائج مقارنة مع المجموعة الضابطة بأن عنصري الــ Li^{+1} , Ni^{+2} أديا إلى ارتفاع معنوي ملحوظ في إنزيمات GpX , CAT , GR والى انخفاض معنوي في مستوى GST بينما سبب عنصر Cs^{++} زيادة في نشاطية GpX , CAT, GST وانخفاض في نشاطية GR. أيضا أدى عنصر Ba^{+2} إلى زيادة معنوية في CAT , GST ونقصان في GR . أما عنصر Mg^{++} فقد أدى إلى زيادة معنوية في نشاطية GpX, GR, CAT, GST بينما أدى عنصر Mo^{+2} إلى زيادة في إنزيمات GST, CAT, GpX ونقصان GR.

أدت هذه العناصر كذلك إلى زيادة معنوية في مستوى الدهن المؤكسد (MDA) . أما حامض الجاليك ومستخلص إكليل الجبل إدي إلي خفض نسبة الدهن المؤكسد MDA بنسب تتراوح ما بين ٢٨-٦٤% و ٩- ٤٨%.

تظهر هذه الدراسة أن عناصر أحادية و ثنائية الاستقطاب لها دور في زيادة الأكسدة وكذلك تأثير واضح لمضادات الأكسدة مثل حامض الجاليك ومستخلص إكليل الجبل في خفض عمليات الأكسدة في كريات الدم الحمراء.

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