THE EFFICIENCY OF SALIVA OFFICINALIS TO REDUCE THE BIOCHEMICAL AND IMMUNOLOGICAL EFFECTS INDUCED BY MANGANESE CHLORIDE IN RATS

Ihsan Raisan Ibrahim
Department of Clinical and Laboratory Sciences, College of Pharamcy, Al Qadisiya University, Iraq
Jameel Kareem Wali
Department of Biology, College of Education, Al- Qadisiya University, Iraq

Abstract

This study was designed to evaluate the role of *Salvia officinalis* extract (sage tea) to reduce the acute effects of manganese chloride $Mncl_2$ in some biochemical and immunological parameters. So, thirty female rats were divided into five groups, first group considered as a control group and given drinking water, the other four groups were injected intraperitoneally with $Mncl_2$ (for ten consecutive days) while sage tea was given instead of drinking water to third, fourth and fifth group at different concentrations for ten days.at the end of the experiment, rats were sacrificed and samples of blood collected for measurment biochemical parameters that included (ALT, AST, ALP, Creatinine and Urea), as well as immunological parameters (Total and differential white blood cells count and phagocytic index).Results showed significant increase ($P<0.05$) in the levels of ALT, AST, ALP, creatinine and Urea) in group injected with $Mncl_2$ only in comparison with control group, as well as there was significant increase in total count of cells, neutrophil, lymphocyte and phagocytic index compared with a control group.Treatment with sage tea and $Mncl_2$ led to significant decrease ($P<0.05$) in levels of ALT, AST, ALP, creatinine and urea in comparison with group treated with $Mncl_2$ only in addition to significant decrease in total white blood cells count, neutrophil and phagocytic index as well as significant increase in monocyte.Its concluded that sage tea have positive effect in reduction the effects of $Mncl_2$.

Keywords: *Salvia officinalis*, manganese chloride, biochemical parameters, WBC, female rats

Introduction

The demand of the world to take health benefits of medical plants is increasing in last years (AL- Eed,2007),sage (*Salvia officinalis*) considered one of these plants which has important therapeutic advantage, Sage leaves use to treat dyspepsia and as bile diuretic and anti-inflammatory (Abu-Zaid,2000). Sage tea used for treatment of many skin and mouth diseases, dyspnea and fever (Dweek and Kintzois ,2000).It was found that the administration of alcoholic extract activates memory in rats and has positive effect in the treatment of ALzheimer disease (Eidi *et al*.,2006; Mehan *et al*., 2011) hypoglycemic effect in experimental diabetic rats (Eidi *et al*., 2005) and as anti-cancer (Keshavarz *et al*.,2011) and anti-mutagenic against mutation induced by UV (Vujosevic *et al*.,2005).Manganese is one of the rare materials that exist in nature in small quantities there are two types of manganese compounds in environment surrounding us, the first inorganic manganese compounds used in the production of steel, batteries and ceramic , and these compounds resulted from engines combustion of cars and factories (Keen and Lonnerdal ,1995). The second type are compounds of organic manganese which used in some pesticides and disinfectants, add to that the compounds of manganese present as minute dust in the air, which in turn can dissolve in ground water, drinking water and reach humans, in addition that manganese can enter the body by inhalation, mouth and skin (Iregren ,1999).

Manganese is one of essential minerals which the human body need them with small quantities and certain limits, if the level of Mn was declined in the body negative effects resulted in public health (Prohaska,1987), in contrast exposure to high levels of Mn can be harmful to health, the high concentration in the body may lead to manganese toxicity. The main target organ is the brain followed by other organs in the body, high accumulation of Mn in brain cause permanent damage and symptoms as difficulty in neuromuscular control, general weakness, mental and emotional disorders and difficulties in breathing and swallowing (Elder *et al*.,2006).Exposure to high doses of manganese leads to reduce the male's fertility in laboratory animals and performed to birth defects in subsequent generation, such as palate cleft and the decrease in the evolution of bone growth. Manganism is term that indicate group of symptoms associated with exposure to relatitively high doses of Mn that include difficulty in breathing and swallowing, as well as neurological problems, symptoms above are similar to symptoms of Parkinson disease (Mergler *et al*., 1999).

Sage contains many components found in genus *salvia* that include monotrepens, ditrepens, flavonoids and phenolic acids (Guan- hua *et al*.,2004). Rasmarinic and carnosic acids are the main phenolic acids compounds in *Salvia officinalis* (Lu and Foo ,1990), also sage contain

volatile oils, saponins, saliva tannins and estrogenic compounds (Dogan ,2004). Thujon, camphore and barnrol are volatile oils in *Salvia officinalis* (Lima *et al*.,2005), In addition there are vitamins (E, C) and some important minerals such as (Ca, K) (Dondivc *et al*.,2001).In this study *Salvia officinalis* was selected in order to evaluate the ability of extract to resist and reduce the negative effects particularly liver enzymes, kidney products(creatinine and urea) and some immunological parameters (phagocytic index , total and differential white blood cells count).

Materials and methods

Laboratory animals

Thirty albino female rats weighting between (182-193) gm and with age (9-11) weeks, housed under normal condition of temperature (23-25) °C and light/ dark (12:12) hr , the animals were fed with pellet diet and drinking water ad libitum.

$Mncl_2$ preparation

The concentration of $Mncl_2$ (20 mg/kg) solution was prepared by dissolving $Mncl_2$ in distilled water and that concentration was selected depending on (Atessahin *et al*.,2003).

Salvia officinalis extract

Sage tea was prepared by adding 2mg of sage leaves powder in 150 ml distilled water and left for 10 minutes (Lima *et al*.,2005). The quantity of sage leaves powder was replicated to obtain the other two concentrations.

Experimental design

Thirty female rats were divided into five groups as following:-

1- Control group animals were injected intraperitoneally with 0.1ml distilled water once daily for ten consecutive days and administrated the normal drinking water along the period of the experiment.
2- First treatment group (T1): animals were injected intraperitoneally with 0.1 ml $Mncl_2$ solution once daily for ten consecutive days and administrated the normal drinking water along the period of the experiment.
3- Second treatment group (T2): animals were injected intraperitonal with 0.1ml $Mncl_2$ solution once daily for ten consecutive days and were administrated the aquatic extract of *Salvia officinalis* with concentration (2mg/150ml) as substitute for drinking water along the duration 0f experiment.
4- Third treatment group (T3): animals were injected intraperitoneally with 0.1 ml $Mncl_2$ solution once daily for ten consecutive days and

were administrated the aquatic extract of *salvia officinalis* with concentration (4mg/150ml) as substitute for drinking water along the duration of experiment.

5- Fourth treatment group (T4): animals were injected intraperitoneally with 0.1ml $Mncl_2$ solution once daily for ten consecutive days and were administrated the aquatic extract of *Salvia officinalis* with concentration (8mg/150ml) as substitute for drinking water along the duration of experiment.

Animals sacrificing

After 24 hours from the last injection animals were sacrificed by exposing them to the inhalation of chloroform, then blood samples were collected by heart puncture, one ml of blood was added in tubes non-containing anticoagulant for 30 minutes, then serum was isolated by centrifuge for 15 minutes at 3000 rpm for obtaining blood serum. Serum stored at temperature -20◦C until performance of laboratory tests.

Biochemical tests

a- Determination of Glutamic oxaloacetate transaminase (AST) activity in serum: Enzymatic method was used to measure the activity of AST which including the use of kit produced by British Randox company, the method is based on the ability of enzyme to work on substrate (Aspartic acid and ɑ- ketoglutaric) and measure the color intensity which is in proportional to the concentration of the enzyme AST (Annino and Giese ,1979).

b- Determination of Glutamate pyruvate Transaminase ALT in serum: ALT was measured depending on enzymatic method (Annino and Giese,1979) and the use of kit produced by Randox company (British).

The principle of this method depend on the activity of ALT on the substrate (Alanine acid and ɑ- ketoglutaric acid), the intensity of color was measured at wave length 546nm.

c- Determination of Alkaline phosphatase (ALP) activity in blood serum:

Enzymatic method was used to evaluate the activity of enzyme ALP (Belfeld and Goldberg ,1971) this method include the addition of phenylphosphates as a substrate for enzyme.

d- Determination of uric acid in blood serum: Uric acid was measured by using phosphotungstic method (Fossati *et al.,* 1980).

e- Serum creatinine: Serum creatinine was measured by a colorimetric endpoint method with deproteinzation (Jaffe reaction) using a kit purchashed from Randoox (UK). In this method creatinine in alkaline

solution reacts with pierate to form colored complex (Burtis and Ashwood , 1999).

Immunological testes

a- Total and differential total white blood cells were measured by following steps described by (Dacie and Lewis, 1984).

b- Phagocytic index :

Phagocytic activity for polymorphonuclear cells in peripheral blood was studies according the method of (Metcalf *et al*.,1986) by adding 0.25 ml of blood in test tube then add 0.15 ml of yeast suspension the tube was incubated at temperature 37°C, after 15, 30, 45 and 60 minutes blood smears was prepared and colored with Leishmania stain and then examined by the light microscope.

Phagocytic was calculated according to the following equation:

$$\text{Phagocytic index} = \frac{\text{Count of yeast phagocytic cells}}{\text{Total count of cells}}$$

Results

Biochemical parameters

Results showed significant increase ($P< 0.05$) in the levels of ALT, AST, ALP in group treated with $Mncl_2$ (T1) in comparison with a control group, on the other hand significant decrease ($P< 0.05$) in enzymes (ALT, AST) in groups treated with $Mncl_2$ and *Salvia officinals* extract (T3, T4) compared with group T1 while there was non significant changes in the levelsof ALT and AST between T2 and T1 groups. ALP reduced significantly ($P< 0.05$) in all groups treated with the extract (T2, T3, T4) compared with group T1 (Table, 1).

Table (2) revealed significant increase ($P< 0.05$) in creatinine and urea levels in groupT1 compared with a control group while there was significant decrease ($P< 0.05$) in the levels of creatinine and urea in groups treated with the extract and $Mncl_2$ (T2, T3 and T4) in comparison with group T1.It was observed there were non-significant changes in the levels of AST, ALT, ALP, creatinine and urea in group T4 compared with a control group.

Immunological tests

Table (3) demonstrate significant increase ($P< 0.05$) in total white blood cell count in group T1 compared with a control group while in groups T2, T3 and T4 there was significant decrease ($P<0.05$) compared with a control group, on the other hand, there was significant increase among the groups T2, T3 and T4 in proportional to the increase in the concentration of

sage extract.Also results in Table (3) showed significant increase (P< 0.05) in neutrophil and phagocytic index in group T1 in comparison with a control group while in groups T3 and T4 which treated with $Mncl_2$ and extract there was significant decrease in neutrophil and phagocytic index compared with group treated with $Mncl_2$ only (T1).Lymphocyte and monocyte percentage decreased significantly (P<0.05) in group T1 compared with a control group, Lymphocyte wasn't affected significantly in groups T2, T3 and T4 compared with group T1 while monocyte percentage elevated significantly in groups T2, T3 and T4 compared with group T1.

Table (1) Effect of manganese chloride and concentrations of aquatic extract of *Salvia officinalis* on the activity of liver enzymes in rats.

Groups / parameters	C	T1	T2	T3	T4
AST (U/L)	**1.19±78.9 a**	**2.34±110.3 c**	**2.44±105 c**	**3.02 ±91.2 b**	**1.22 ±81.3 a**
ALT (U/L)	**0.49 ±30.6 a**	**0.64 ±41.2 c**	**0.8 ±39.1 c**	**1.17 ±35 b**	**0.82 ±31.8 a**
ALP (U/L)	**3.03 ±116.5 a**	**1.72 ±154.1 d**	**1.42 ±140.3 c**	**2.40 ±125 b**	**2.58 ±118.9 ab**

Numbers refer to Mean ± SE

a,b,c,d :different letters within the same row indicate significant differences (P<0.05) among the groups.

Table (2) Effect of manganese chloride and concentrations of aquatic extract of *Salvia officinalis* on some parameters of kidney function.

Groups / parameters	C	T1	T2	T3	T4
Creatinin mm/l	**0.02 ±0.45 a**	**0.05 ±1.2 d**	**0.05 ±0.91 c**	**0.03 ±0.62 b**	**0.02 ±0.55 ab**
Urea mm/l	**0.64 ±35.2 a**	**0.92 ±52.1 d**	**1.10 ±48.7 c**	**0.59 ±38.3 b**	**0.57 ±37.1 a**

Numbers refer to Mean ± SE

a,b,c,d :different letters within the same row indicate significant differences (P<0.05) among the groups.

Table (3) Effect of manganese chloride and concentrations of aquatic extract of *Salvia officinalis* on some immunological parameters.

Groups / parameters	C	T1	T2	T3	T4
WBC cell/mm^3	**0.38 ±7.4 a**	**0.39 ±15.8 d**	**0.32 ±14.1 c**	**0.25 ±11.5 b**	**0.42 ±8.3 a**
Neutrophil %	**0.61 ±29.8 a**	**0.95 ±34.94 c**	**0.44±33.8 bc**	**0.82±32.78 b**	**0.55±28.9 a**
Eiosnophil %	**0.34±3.2 a**	**0.38±3.62 a**	**0.29±3.56 a**	**0.39±3.8 a**	**0.36±3.1 a**
Lymhpocyte %	**1.54±61.9 b**	**0.98 ±57.14 a**	**0.88±56.7 a**	**1.1 ±58.2 a**	**1.22±60.6 ab**

Monocyte %	**1.01 ±5.28 ab**	**0.95 ±4.36 a**	**1.33±5.6 b**	**1.57±5.82 b**	**1.81±6.7 b**
Phagocytosis index %	**0.36 ±8.5 a**	**0.29 ±13.2 c**	**0.23±12.8 c**	**0.25 ±9.3 ab**	**0.10 ±8.9 ab**

Numbers refer to Mean ± SE.
a,b,c,d :different letters within the same row indicate significant differences ($P<0.05$) among the groups.

Discussion

Biochemical parameters

$Mncl_2$ injected in rats creates toxic effects as demonstrated by marked increase in activities of AST, ALT and ALP enzymes, effects of $Mncl_2$ may related to increase in oxidative stress, $Mncl_2$ stimulate Lipid peroxidation in tissues of rats (Chen *et al*.,2006), also Zhang *et al.* (2003) indicated that $Mncl_2$ elevate the production of reactive oxygen species (ROS) in the mitochondria of rat liver.On the other hand, $Mncl_2$ at large doses caused renal damage in rat (Atessahin *et al.*,2003) and this agreed with results of the current study as indicated by the increase in the levels of urea and creatinine.

The role of *Salvia officinalis* in improvement biochemical parameters due to antioxidant activity of *Saliva officinalis*. Oboh and Henle (2009) found that the aqueous extract of *Salvia officinalis* inhibit the product of lipid peroxidation Malondialdehyde (MDA) in brain and liver of rats, also *Salvia officinalis* extract caused significant increase in glutathione -S- transferase and glutathione reductase in rat liver (Lima *et al*.,2005), the protective effect of *Salvia officinalis* decrease the release of enzymes ALT, ASTand ALP from hepatocytes and then reduce their levels in blood.Also Lima *et al*.(2005) showed that *Salvia officinalis* cause significant increase in glutathione -S- transferase and glutathione reductase in rats, as well as *Salvia officinalis* extract elevate the activity of superoxide dismutase (SOD), Catalase (CAT) and glutathione (GSH) (El- kholy *et al*.,2010), *Salvia officinalis* have hepatoprotective effect against azathioprine that induced hepatotoxity in rats (Amin and Hamza ,2005).The antioxidant activity of *Salvia officinalis* correct the effects induced by $Mncl_2$ in the levels of AST, ALT, ALP, urea and creatinine and the decrease in these parameters was dose dependent effect.

Immunological parameters

$Mncl_2$ caused significant increase in WBCs, neutrophils and phagocytic index and these effects may be resulted from oxidative stress induced by the increase in ROS formation. $Mncl_2$ stimulate the generation of ROS in vitro (Zhang *et al.*,2004).On the other hand, oxidative stress may cause chronic inflammation (Reuter *et al.*,2010). intramuscular injection

with $Mncl_2$ caused increase in phagocytic activity in mice (Smialowics *et al.*,1985).Active components isolated from *Salvia officinalis* causes decrease formation of stimulating substances for WBCs formation which called Leukotriens resulted in decrease the activity and numbers of WBCs in human (Poecked *et al.*,2008).The decrease in WBC may be resulted from role of *Salvia officinalis* in inhibit cells migration, Jedinak *et al*.(2006)found that *Salvia officinalis* inhibit endothelial cell migration in the process of engiogenesis and this effect was indicated by the presence of B- ursolic as active compound in *Salvia officinalis* extract.On the other hand *Salvia officinalis* extract increase the activity of antioxidant system such as GSH and GST which are important antioxidants that prevent the damage by ROS (Lu and Foo ,1990; Carla *et al*.,2009).

Conclusion

It was concluded that *Salvia officinalis*(sage tea)declined the negative effects of manganese chloride due to it's antioxidant activity against the harmful effects of manganese chloride.

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