LEAD TOXICITY AND THE PROTECTIVE ROLE OF Cupressus sempervirens SEEDS GROWING IN EGYPT

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ABSTRACT

The toxicological effect of lead acetate to the liver and the possible protective effect of Cupressus sempervirens (C. sempervirens) plant and its flavonoids (quercetin and rutin) was evaluated. 30 Male albino rats and divided into five groups (six per group). Group I, served as control, group II exposed to 0.5 mg/g concentrations of lead acetate in diet for 60 days. Group III was received daily doses of 8 mg/100g of rat b.wt of C. sempervirens (liophilized from methanol extract of seeds) two weeks prior to lead acetate administration. Group IV received daily doses of 0.3 mg/100g of rat b.wt of the flavonoid quercetin two weeks prior to lead acetate administration; Group V was received daily doses of 0.1 mg/100g of rat b.wt of the flavonoid rutin two weeks prior to lead acetate administration. Lead acetate caused a significant increase in serum and tissue AST, ALT, ALP, bilirubin, serum and tissue MDA, plasma and tissue NO, in addition to, highly significant increase in serum cholesterol, LDL, triglycerides and HDL. On the contrary, lead induced a significant decrease in serum and tissue total protein, albumin, globulin, albumin/globulin ratio, blood and tissue SOD and GPx compared to control group. The administration of C. sempervirens methanol extract, quercetin and rutin two weeks prior to lead acetate prevents these parameters to the normal levels. In conclusion, the treatment with C. sempervirens methanol extract and its flavonoids may provide a partial protection against the toxic effect induced by lead acetate. This indicates that C. sempervirens methanol extract may be capable of greatly modifying rodents’ susceptibility to blood and liver toxicity in addition to oxidative stress induced by lead acetate.

Keywords: Cupressus sempervirens, Cupressaceae, quercetin, rutin, lead toxicity.

RESUMEN

El presente estudio se condujo para elucidar el posible efecto protector de C. sempervirens y sus flavonoides (quercetina y rutina) en caso de intoxicación de acetato de plomo en ratas albinas. Los resultados en el grupo de ratas intoxicadas con plomo revelan toxicidad en el hígado, estrés oxidativo e hipercolesterolemia. El grupo tratado con C. sempervirens y sus flavonoides (quercetina y la rutina) evita los efectos tóxicos de la exposición al plomo.

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LIST OF ABBREVIATIONS

NRC: National Research Centre; C. sempervirens: Cupressus sempervirens; ANOVA: Analysis of Variance; AST: Aspartate Aminotransferase; ALT : Alanine Aminotransferase; ALP: Alkaline Phosphatase; Alb: Albumin; Glob: Globulin; MDA: Malondialdehyde; NO: Nitric Oxide; LDL: Low Density Lipoproteins; HDL: High Density Lipoproteins; SOD: Superoxide Dismutase; GPx: Glutathione Peroxidase; LD50: Lethal Dose Fifty; B.wt.: Body Weight

INTRODUCTION

C. sempervirens (Fig. 1) belongs to the family Cupressaceae (Hortus, 1976). C. sempervirens is rich in flavonoids constituents such as cupressuflavone, amenoflavone, rutin, quercitrin, quercetin, myricitrin (Harborne, 1993). Some of phenolic compounds (anthocyanidin, catechines flavones, flavonols and isoflavones) tannins (ellagic acid, gallic acid, phenyl isopropanoids, caffeic acid, coumaric acid, ferulic acid) lignans, catchol. essential oil of Egyptian C. sempervirens cones contains 49 components with a-pinene (48.2%) and 3-carene (19.1%) as the main components (Kassem et al., 1991). The cones and young branches are anthelmintic, antipyretic, anti-rheumatic, antiseptic, astringent, balsamic, vaso-constrictive, anti-inflammatory, hair tonic, the fruits of the plant were used traditionally for curing diabetes and as antiseptic (Said et al., 2002).

Taken internally it used in the treatment of whooping cough, the spitting up of blood, spasmodic, cough, cold, flu and sore throats, while applied externally as a lotion or a diluted essential oil (using an oil such as almond), it astringes varicose veins and hemorrhoids, tightening up the blood vessels, A foot bath of the cones is used to cleans the feet and answers excessive (Chevallier, 1996).

An essential oil from the leaves and cones is used in aromatherapy. Its key word is Astringent (Westwood, 1993). There are other uses of C. sempervirens like: cosmetic
(an essential oil distilled from the shoots is used in perfumery and soap making (Usher, 1974).

Lead (Pb), a highly toxic heavy metal, is widely distributed in nature. Pb is the most ancient poison known to man. Contamination of soil, water and air has become increasingly widespread through mining, refining and smelting operations. The concentrations of Pb in liver and bone of red deer and wild boar were higher in the mining area than in the control area (Reglero et al., 2009). The inhibitory effect of dietary Pb on digestive enzyme activities was dietary Pb concentration dependent (Dai et al., 2009).

Metallothionein (MT) is a low-molecular-weight cysteine-rich protein which has a high affinity for metals and plays important roles in the protection against metal toxicity. Administration of Pb increased the levels of MT-I mRNA in the liver, Where, Pb enhanced MT gene transcription gene in the liver (Yu et al., 2009).

The aim of this study was to ascertain the possible protective effect of C. sempervirens plant and its flavonoids (quercetin and rutin) in lead acetate treated group for this reason biochemical, histo-pathological and DNA content were done.

MATERIALS AND METHODS

Chemicals
Lead acetate was obtained from E. Merck, while the flavonoids (quercetin and rutin) were purchase from Sigma LTD Company as a pure powder compound.

Basal diet containing lead
Rats were fed lead-basal diet diet-mixes prepared with purified rat basal (95%) Diet mix (Production Code TD 621, powder) obtained from food toxicology and contamination department, Faculty of Agriculture, Cairo University, Egypt. Each diet contained complete 100% normal nutrition components, when the diet was diluted to 95% with 5% (w/w) bulk test agents, i.e., basal diets containing lead. Lead acetate was dissolved into a vehicle of sterile, deionized H₂O and evenly atomized in one-third portions over the surface of the diet mix and then blended for an additional 15 min. Therefore, the effective diet lead concentration was 0.5 mg lead/g diet (Smith et al., 2009).

Experimental animals and treatments
30 Male albino rats were used for this experiment. The animals were kept under conventional conditions (temperature 20-22 °C, humidity 60-70%, 12 h light: 12 h dark cycle) and fed with standard rodent chow. Food and water were available at all times. The experiments were carried-out in accordance with the national regulations on animal welfare and Institutional Animal Ethical Committee (IAEC), “National Research Centre (NRC), Cairo – Egypt” (Permission no.213). The albino rats were obtained from the animal house at NRC, Cairo- Egypt and housed in polypropylene cages. Rats were 10 weeks-old at the onset of the experiments and had an average weight of 100±10g.

The animals were divided into five groups (six per group). Group I, served as control and received the same volume of distilled water, group II was exposed to 0.5 mg/g concentrations of lead acetate in diet for 60 days (Moridani et al., 2003). Group III received daily 1/10 LD₅₀ (8mg/100g of rat b.wt dissolved in 1ml distilled water) of C. sempervirens methanol extract through stomach tube two weeks prior to lead acetate administration. Group IV taken daily 1/10 LD₅₀ (0.3mg/100g of rat b.wt of the flavonoid quercetin dissolved in 0.5ml) through stomach tube two weeks prior to lead acetate administration (Koriem et al., 2005). Group V was administrated daily 1/10 LD₅₀ (0.1mg/100g of rat b.wt of the flavonoid rutin dissolved in 0.5ml) through stomach tube two weeks prior to lead acetate administration (Koriem et al., 2005).
Lead toxicity and the protective role of *Cupressus sempervirens*

**Plant material**
Seeds of *C. sempervirens* were collected (7 kilograms) from National Research Center Garden, Cairo, Egypt. Voucher seeds of each plant were deposited at the herbarium of the National Research Centre.

**Extraction and isolation**
The powder of the dried seeds of *Cupressus sempervirens* was defatted with methyl chloride (CH$_3$Cl) and extracted with methanol-water [CH$_3$OH:H$_2$O (7:3)] at room temperature. The methanol extract was filtered, evaporated under reduced pressure and lyophilized (200g). At the time of use, the extract was suspended in distilled water. The extraction process was taken one month from collection of plant seeds until final methyl extract estimated. The purpose of using this medicinal plant is due to its high constituents of flavonoids. Methanol extract was used due to its boiling point is very low, easily evaporated and low in cost. Methyl extract is more common in use than ethyl extract. Although methyl alcohol is toxic, but in the present study the dried part of the employed plant was extracted and the methyl alcohol was evaporated and there was no toxicity in the plants extracts. The chemical constituents of *Cupressus sempervirens* methanol extract was determined using gas chromatography-mass spectra (GC-MS) (Table 1).

**Blood Sampling and Handling**
Blood samples were collected from retro-orbital plexus of rats using capillary tubes (Schermer, 1967) into clean centrifuge tubes. Part of blood sample was collected on EDTA as anticoagulant for SOD and GPx. The other part of the blood sample was allowed to coagulate and centrifuged at 4000 r.p.m. for 15 minutes to separate blood serum. Separated serum was stored at -20°C for the determination of liver function [AST, ALT, bilirubin, ALP, total protein, Alb, Glob and Alb/Glob ratio]. Total cholesterol, LDL-cholesterol, trigly-ceride and HDL-cholesterol were measured also in serum.

**Prepared of liver tissue homogenate**
After collection of the blood, the animals were decapitated and then dissected whereby the liver was obtained, washed in cold saline and dried between filter papers. The liver was weighed, homogenized and kept at -20°C for further investigation. 0.5 mg of liver tissue was dissolved in 2.5 ml of tris buffer solution, then homogenate in the homogenizer for exactly 30 minutes. Then centrifuge for exactly 20 minutes at 7,000 r.p.m. Then separate the supernatant, which proceed in the same manner of blood serum.

**Determination of LD$_{50}$ of the methanol extract**
The LD$_{50}$ of the methanol extract of *C. sempervirens* was determined in mg/kg body

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**Table 1:** Chemical constituents of *Cupressus sempervirens* Methanol extract.

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Glycoside</th>
<th>Tannins</th>
<th>Coumarine</th>
<th>Unsat. st.</th>
<th>Leu.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>Flavone</td>
<td>Rutin</td>
<td>Catechines</td>
<td>Flavonols</td>
<td>LB</td>
</tr>
<tr>
<td>(+++)</td>
<td>(+)</td>
<td>(+++)</td>
<td>(+)</td>
<td>(+)</td>
<td>++</td>
</tr>
</tbody>
</table>

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weight for adult albino rats (Behren and Karber, 1953). Where, LD$_{50}$ = DM-$\Sigma$Z.d/m
DM= the highest dose used, Z= the number of dead rats of two successive doses
divided by two, d= the difference between
two successive doses, m= the number of
rats in each group.

A dose of 1/10 of the LD$_{50}$ of each ex-
tract seems to be safe in animals (Arbid et al., 2006).

**Histopathological examinations**
Specimens of liver was fixed in 10% neutral
formalin solution, and then processed for
routine technique for embedding in para-
fin. Blocks were sectioned at a thickness
of 5μm and stained with haematoxylin and
eosin for histopathological examination.

**BIOCHEMICAL ESTIMATIONS**

**Liver Function**
Serum transaminases (AST and ALT) were
determined (Reitman and Frankel, 1957).
Serum total bilirubin determination was
performed (Walter and Gerarde, 1970). ALP
was determined (Kind and King, 1954). The
determination of serum total protein was
performed (Gornall et al., 1949). Deter-
nmination of serum Alb was done (Drupt,
1974). Serum Glob and Alb/Glob ratio were
estimated (Latner, 1975).

**Lipids Fractions**
Total cholesterol was determined (Allain
et al., 1974) using enzymatic method.
Determination of serum LDL was adopted
(Steinberg, 1981). Serum triglycerides were
determined (Fossati and Prencipe, 1982). Serum HDL was determined (Fruchart et
al., 1982).

**Antioxidant enzymes**
Determination of SOD in blood was esti-
imated (Suttle, 1986). Blood GPx was es-
timated (Pagalia and Valentine, 1967). A
colorimetric assay was used for detecting
MDA in serum (Esterbauer et al., 1991).
Plasma NO was analyzed (Moshage et al.,
1995).

**Image Analysis:**
Feulgen-stained slides were prepared for
the nuclear DNA analysis at the Pathology
Department, National Research Center
using the Leica Qwin 500 Image Analyzer
(LEICA Imaging Systems Ltd, Cambridge,
England). The system was calibrated be-
fore each measurement session using the
calibration slides that provided with the
system at high power magnification (400X).
The optical density of the selected nuclei in
each microscopic field was measured and
automatically converted by the system into
DNA content.

The results were displayed as a fre-
quency histogram on the monitor gener-
ated by plotting the DNA content versus
the number of nuclei counted. The DNA
histograms were classified as diploid (DNA
index ranging from 0.9 to 1.1), tetraploid
(DNA index ranging form 1.8 to 2.2) and
aneuploid (when at least 10% of the total
events show a distinct abnormal peak
outside the 2C or 4C position) based on
the amount of DNA relative to the normal
control (Danque et al., 1993).

**Statistical analysis**
Data were evaluated with program of Sta-
tistical Package for Social Sciences/Ver-
sion 10 (SPSS/10) software for Windows.
Hypothesis testing methods included two
way analysis of variance (ANOVA) followed
by least significant difference (LSD) test
at P<0.0001. P values of less than 0.05
were considered to indicate statistical sig-
nificance. Comparisons of multiple groups
were done and Data were tabulated. Results
were expressed as mean ± SD and signifi-
cant difference according to –ve (control)
and +ve (lead) control groups.
RESULTS

Biochemical Results

The lethal dose fifty (LD50) of *C. sempervirens* methanol extract was 800 mg/kg body weight (table 2).

The results presented in table 3 indicated that lead acetate caused a highly significant increase (P<0.01) in serum AST, ALT and bilirubin, also, significant increase (P<0.05) in serum ALP. Lead caused a significant decrease (P<0.05) in serum protein, Alb and Glob and highly significant decrease (P<0.01) in Alb/Glob ratio. Administration of *C. sempervirens*, quecetin and rutin avert the toxic effects of lead exposure and decrease serum AST, ALT, ALP and bilirubin, as well increase serum protein, Alb, Glob and Alb/Glob ratio was found compared to the lead treated rats.

The analysis of the data found in table 3 indicated that lead acetate caused a highly significant increase (P<0.01) in liver Alb/Glob ratio, while, highly significant decrease (P<0.01) in tissue total protein and Alb. Tissue Glob recorded a highly significant decrease (P<0.01) in tissue Glob. Administration of *C. sempervirens*, quecetin and rutin prevent the toxic effects of lead exposure, where decrease in liver AST, ALT and Alb/Glob ratio, but increase liver protein, Alb and Glob was observed compared to the lead treated rats.

### Table 2: Acute oral lethal toxicity of dried methanol extract of *Cupressus sempervirens*

<table>
<thead>
<tr>
<th>GP No.</th>
<th>Dose (mg/kg b.wt.)</th>
<th>No. of Animals /gp</th>
<th>No. of dead animals</th>
<th>Z</th>
<th>D</th>
<th>Z.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>780</td>
<td>10</td>
<td>0</td>
<td>0.5</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>785</td>
<td>10</td>
<td>1</td>
<td>1.5</td>
<td>5</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>790</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>795</td>
<td>10</td>
<td>4</td>
<td>4.5</td>
<td>5</td>
<td>22.5</td>
</tr>
<tr>
<td>5</td>
<td>800</td>
<td>10</td>
<td>5</td>
<td>5.5</td>
<td>5</td>
<td>27.5</td>
</tr>
<tr>
<td>6</td>
<td>805</td>
<td>10</td>
<td>5</td>
<td>5.5</td>
<td>5</td>
<td>27.5</td>
</tr>
<tr>
<td>7</td>
<td>810</td>
<td>10</td>
<td>6</td>
<td>6.5</td>
<td>5</td>
<td>32.5</td>
</tr>
<tr>
<td>8</td>
<td>815</td>
<td>10</td>
<td>7</td>
<td>7.5</td>
<td>5</td>
<td>37.5</td>
</tr>
<tr>
<td>9</td>
<td>820</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>5</td>
<td>45</td>
</tr>
</tbody>
</table>

LD50 = Dm-∑ (Z.D)/n
= 820-19 = 801≈800 mg/kg b.wt.

It was found that a significant decrease (P<0.05) in blood SOD and GPx were recorded with lead acetate. The administration of *C. sempervirens*, quecetin and rutin to rats taken lead acetate avert the toxic effects of lead exposure and induce increase in blood SOD and GPx when compared with that received lead acetate only. Lead acetate caused significant increase (P<0.05) in serum MDA and plasma NO compared with the normal rats. On the other hand, *C. sempervirens*, quecetin and rutin administration prevent the toxic effects of lead exposure and increase blood SOD and GPx but decrease serum MDA and plasma NO was recoded compared with that treated with lead acetate alone (table 4).

The results exhibited in table 4 showed that lead acetate caused significant decrease (P<0.05) in liver SOD, GPx and MDA but highly significant increase (P<0.01) in liver NO was occurred compared to control rats. Administration of *C. sempervirens*, quecetin and rutin avert the toxic effects of lead exposure and increase liver SOD, GPx and MDA, while decrease plasma NO was found compared to lead acetate treated rats.
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Lead acetate induced significant increase (P<0.05) in serum cholesterol, LDL, triglycerides and HDL was reported compared to control rats, in the contrary, C.sempervirens, quecetin and rutin administration to rats taken lead acetate prevent the toxic effects of lead exposure and decrease serum cholesterol, LDL, triglycerides and HDL was happened compared to lead feeding rats (table 4).

DNA cytometry results
The liver of control group showed 65.7 % (2C) diploid cells, 11.7 % (3C) triploid cells (medium proliferation index), 0.9 % (4C) tetraploid cells and 0 % (>5C) aneuploid cells (diploid-medium proliferation index) [table 5].

In lead acetate treated group, the liver showed 3.7 % (2C) diploid cells, 9.4 % (3C) triploid cells (low proliferation index), 24.5 % (4C) tetraploid cells and 36.7 % (>5C) aneuploid cells (aneuploid-low proliferation index) [table 5].

In rats treated with lead acetate and C.sempervirens or the flavonoids (quecetin or rutin), the liver showed 40.0 % (2C) diploid cells, 39.0 % (3C) triploid cells (high proliferation index), 15.4 % (4C) tetraploid cells and 0.9 % (>5C) aneuploid cells (diploid-high proliferation index) [table 5].

These results showed that lead acetate caused very high increase (aneuploidy) in the DNA content of the liver cells, put not reaching a neuploidy. With treatment C.sempervirens or the flavonoids (quecetin or rutin) prevented the toxic effects of lead exposure and the DNA content of liver cells and approach but not to the normal value.

Histo-pathological Results
The liver composed of hepatic lobules. Each lobule consists of a plate of parenchymal cells or hepatocytes. Slits-like spaces or blood sinusoids are found between the hepatocytes. The blood sinusoid extends from the periphery of the hepatic lobule to the central vein. The hepatocytes nuclei are large in size, rounded and bounded by dis-
Table 4: Blood and liver antioxidants, in addition lipid profile of rats treated with Cupressus sempervirens plant or its flavonoids (quercetin and rutin) and lead acetate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (-ve control)</th>
<th>Lead Acetate (+ve control)</th>
<th>Treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. sempervirens+ Lead acetate</td>
</tr>
<tr>
<td>Blood SOD (U/ml)</td>
<td>260 ± 8.39</td>
<td>234.5 ± 7.86</td>
<td>255.0 ± 6.63*</td>
</tr>
<tr>
<td>Blood GPx (U/mL)</td>
<td>6250 ± 82.95</td>
<td>5975 ± 98.71</td>
<td>6250 ± 86.93*</td>
</tr>
<tr>
<td>Serum MDA (µ mol/l)</td>
<td>3.51 ± 0.46</td>
<td>5.01 ± 0.54</td>
<td>3.86 ± 0.28**</td>
</tr>
<tr>
<td>Plasma NO (µ mol/l)</td>
<td>34.5 ± 1.78</td>
<td>41.3 ± 2.25</td>
<td>37.5 ± 1.69</td>
</tr>
<tr>
<td>Liver SOD (U/g)</td>
<td>48.50±4.25</td>
<td>36.25±3.40</td>
<td>45.46±2.89*</td>
</tr>
<tr>
<td>Liver GPx (U/g)</td>
<td>14.15±1.44</td>
<td>10.73±0.92</td>
<td>13.20±0.46*</td>
</tr>
<tr>
<td>Liver MDA (µ mol/g)</td>
<td>29.6 ± 1.87</td>
<td>23.8 ± 1.98</td>
<td>28.9±1.65*</td>
</tr>
<tr>
<td>Liver NO (µ mol/g)</td>
<td>9.70±1.753</td>
<td>21.50±2.32</td>
<td>14.00±2.18*</td>
</tr>
<tr>
<td>Serum Cholesterol (mg/dl)</td>
<td>98.2 ± 3.46</td>
<td>115.1 ± 2.86</td>
<td>104.0 ± 4.05*</td>
</tr>
<tr>
<td>Serum LDL(mg/dl)</td>
<td>40.7 ± 1.95</td>
<td>49.3 ± 1.86</td>
<td>44.5 ± 2.04</td>
</tr>
<tr>
<td>Serum Triglycerides (mg/dl)</td>
<td>47.1 ± 2.01</td>
<td>55.0 ± 1.93</td>
<td>51.8 ± 2.14</td>
</tr>
<tr>
<td>Serum HDL (mg/dl)</td>
<td>48.1 ± 1.87</td>
<td>54.8 ± 2.03</td>
<td>49.1 ± 1.92*</td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SD and significant difference according to control group at P<0.05, ANOVA showed a highly significant difference between all groups at P<0.0001. *P<0.05 significant difference, **P<0.01 highly significant differences and ***P<0.001 very high significant differences compared to -ve y +ve controls.

Table 5: DNA Ploidy livers of the control, lead acetate and C. sempervirens then lead acetate.

<table>
<thead>
<tr>
<th>Range</th>
<th>Total Cells</th>
<th>% Cells</th>
<th>DNA Index</th>
<th>Range</th>
<th>Total Cells</th>
<th>% Cells</th>
<th>DNA Index</th>
<th>Range</th>
<th>Total Cells</th>
<th>% Cells</th>
<th>DNA Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>111</td>
<td>100.0%</td>
<td>1.000</td>
<td>All</td>
<td>106</td>
<td>100.0%</td>
<td>2.374</td>
<td>All</td>
<td>107</td>
<td>100.0%</td>
<td>1.923</td>
</tr>
<tr>
<td>5cER</td>
<td>0</td>
<td>0.0%</td>
<td>-</td>
<td>5cER</td>
<td>39</td>
<td>36.792%</td>
<td>2.948</td>
<td>5cER</td>
<td>11</td>
<td>10.28%</td>
<td>2.815</td>
</tr>
<tr>
<td>&lt; 1.5c</td>
<td>24</td>
<td>21.622%</td>
<td>0.679</td>
<td>&lt; 1.5c</td>
<td>0</td>
<td>0.0%</td>
<td>-</td>
<td>&lt; 1.5c</td>
<td>0</td>
<td>0.0%</td>
<td>-</td>
</tr>
<tr>
<td>1.5c-2.5c</td>
<td>73</td>
<td>65.766%</td>
<td>1.031</td>
<td>1.5c-2.5c</td>
<td>4</td>
<td>3.774%</td>
<td>1.045</td>
<td>5cER</td>
<td>6</td>
<td>5.607%</td>
<td>1.181</td>
</tr>
<tr>
<td>2.5c-3.5c</td>
<td>13</td>
<td>11.712%</td>
<td>1.359</td>
<td>2.5c-3.5c</td>
<td>10</td>
<td>9.434%</td>
<td>1.558</td>
<td>2.5c-3.5c</td>
<td>35</td>
<td>32.71%</td>
<td>1.595</td>
</tr>
<tr>
<td>3.5c-4.5c</td>
<td>1</td>
<td>0.901%</td>
<td>1.779</td>
<td>3.5c-4.5c</td>
<td>26</td>
<td>24.528%</td>
<td>2.033</td>
<td>3.5c-4.5c</td>
<td>46</td>
<td>42.991%</td>
<td>1.966</td>
</tr>
<tr>
<td>&gt; 4.5c</td>
<td>0</td>
<td>0.0%</td>
<td>-</td>
<td>&gt; 4.5c</td>
<td>66</td>
<td>62.264%</td>
<td>2.712</td>
<td>&gt; 4.5c</td>
<td>20</td>
<td>18.692%</td>
<td>2.622</td>
</tr>
</tbody>
</table>
distinct nuclear envelop. The structure of the control liver showed normal hepatocytes, vascular sinusoids and centrolobular vein (Fig. 2-A).

Administration of lead acetate (0.5 mg/g diet) for 60 days caused a hoop of oedema in the peri-portal area, which compressed the surrounding hepatocytes. Notice, the intracytoplasm vaculation was found (Fig. 2-B).

Administration of *C. sempervirens* and quercetin flavonoid prior to lead acetate revealed that the hepatocytes are within normal limit and preserved its plate pattern. Liver almost prevents to the normal pattern (Fig. 2-C y D).

**DISCUSSION**

LD$_{50}$ of *C. sempervirens* methanol extract was 800 mg/kg b.wt, comparing with that of Karkabounas *et al.* (2003) was 400mg/kg b.wt for aqueous-methanol extract, and Ibrahim *et al.* (2007) was 750mg/kg b.wt for *C. sempervirens* methanol extract.

The results revealed that lead acetate induced liver toxicity through increase serum liver enzymes and decrease serum and liver total proteins. The elevation of serum AST and ALT due to the possible release of these enzymes from the cytoplasm, into the blood circulation rapidly after rupture of the plasma membrane and cellular damage and ALP is an ecto-enzyme of hepatocyte plasma membrane; an increase in serum ALP reflects the pathological alteration in biliary flow and damage to the liver cell membrane (Giuliani *et al*., 1983, Dunsford *et al*., 1989). High serum concentrations of serum transaminases are considered to be an index of hepatic injury where eleva-
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Expression of ALT is regarded as a more sensitive indicator and is usually accompanied by a rise in AST (Ha *et al*., 2001). While, the decrease in the level of total proteins was explained by liver injury is associated with decreased albumin level secondary to decreased protein synthesis (Comporti, 1985). Administration of *C. sempervirens*, quecitin and rutin avert liver toxicity induced by lead acetate. The mechanism of protection depends on flavonoids play as phyto-estrogen, which induced aromatase activity in the cells and increased mRNA expression with concurrent elevation in the usage of promoters I.3/II, and several protein kinases were activated (PKC, P38, ERK-1/2) and consequently the transcriptional factor CREB was ultimately activated in the gene regulation, so the protein synthesis is increased consequently (Ye *et al*., 2009). These data coincide with Rizk *et al*., (2007) and Ibrahim *et al*., (2007); they approved that methanol extract of *C. sempervirens* leaves extract exhibited protection against CCL4-induced lethality in rats suggesting hepato-protective action by prevention of CCL4-induced raise in liver enzymes.

Lead toxicity induced oxidative stress by decrease blood and liver SOD and GPx and increase MDA and NO in both serum and liver. The administration of *C. sempervirens*, quecitin and rutin to rats prior to lead acetate exhibited significant protection against oxidative stress induced by lead acetate. The mechanism of protection relay on cellular action of flavonoids and their ability to modulate gene expression and the activity levels of enzymes involved in antioxidant defense and glutathione activity (Wiegand *et al*., 2009). These results are parallel with Moon *et al*., (2006) and Sivaramakrishnan *et al*., (2008) who observed that plant- derived flavonoid is believed to increase SOD and GPx level as anti-oxidative plant and beneficial health effects of flavonoids. *C. sempervirens* leaves methanol extract caused a decrease in the level of nitric oxide (NO) in cirrhotic rats (Rizk *et al*., 2007). The methanol extracts of leaves of male and female, and fruits of all species of *C. sempervirens* possessed antioxidant activity when tested with both the Ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods (Emami *et al*., 2007 and Ibrahim *et al*., 2007).

Lead acetate induced hypercholesterolemia, where lead exposure increased hepatic cholesterogenesis and this accompanied with a decrease in triglyceride and phospholipid contents in the liver and consequently increased in blood (Formica and Regelson, 1995). The administration of *C. sempervirens*, quecitin and rutin to rats prior to lead acetate decrease hypercholesterolemia induced by lead acetate. The mechanism of protection depends on flavonoids protect low density lipoproteins from oxidation (prevent artherosclerotic plaque formation), where lipids targeted for cellular metabolism are mobilized from the intestine as: (1) triglycerides-rich chylomicrons; (2) triglycerides-rich very-low-density lipoproteins, which are subsequently converted to cholesterol-rich LDL; and (3) as cholesterol-phospholipid-rich HDL, which increased moving of cholesterol from peripheral cells and from the liver to the blood (Ahmed *et al*., 2007). This observations are coincides with researchers who observed that oral administration of *C. sempervirens* cone extract (CSE) resulted in a substantial decrease of serum total cholesterol, which was significant after 6 weeks of treatment. Moreover, these animals exhibited lower total cholesterol levels compared to the controls after the initiation of treatment during the study period (Ademuyiwa *et al*., 2009).

Histological examinations in this study showed that lead acetate treated group revealed a loss of hepatic lobular architecture. This is coinciding with many researchers (Yu *et al*., 2008) who observed lesions detected in the Pb-contaminated groups and detected serious damage in the hepatic structure and function in the
groups received basal diet supplemented with lead. The administration of *C. sempervirens* extract and its flavonoids (quercetin and rutin) prevented liver tissue injury approach to normal pattern and this is parallel to that who observed that the hepatic cells, central vein and portal triad are almost normal in *C. sempervirens* methanol extract in CCL4 intoxicated group (Rizk *et al.*, 2007).

In summary, it was demonstrated that *C. sempervirens* methanol extract (1/10 LD50) could produce protective effect in rats intoxicated with lead. This response was reflected on the blood, liver and oxidative toxicity. This may probably occur, in a way or another, to human individuals have been subjected to environmental pollution (like lead toxicity) that may, in turn, lead to blood, liver and oxidative stress toxicity and hence increase the susceptibility to the toxic effects. The pretreatment with *C. sempervirens* methanol extract from seeds gave protective effect against those unfavorable substances, which suggest that using this medicinal plant in therapy is recommended, this effect related to its flavonoids constituents and this result was supported by histological examinations.

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