Protective effects of dietary dates against the toxicity of mercuric chloride in male albino rats
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SUMMARY

The present study was planned to investigate the protective effect of dates on the adverse effect of mercuric chloride toxicity in rats. Sixty rats were divided into four equal groups, group I served as a control, group II fed basal diet and administrated 1/20 LD50 mercuric chloride (2.29 mg/ kg b. w.), group III fed basal diet supplemented with 10% minced date only four weeks before exposure to mercuric chloride and the group IV fed basal diet with 10% minced dates four weeks before exposure to mercuric chloride, both mercuric chloride and dates were continued along the experiment in this group. Referring to hematological parameters, group II and III mercuric chloride induced significant decrease in RBCs count, hemoglobin content and PCV with normocytic normochromic anemia, significant decrease in WBC count but no significant changes were recorded in group IV. Group II showed significant increased in serum sodium, ALT, AST and urea while potassium, total protein, albumin, globulin and testosterone showed significant decrease in rat serum. Significant increase of serum creatinine was detected at 90 days, the same results were showed in group III but in less density except creatinine showed non significant changes. In group IV, serum sodium potassium, creatinine and testosterone revealed no significant changes, but serum ALT, AST, total protein, albumin, globulin and urea were largely improved. Mercuric chloride was found to decrease phagocytotic percentage and phagocytic index of neutrophils in rats received mercuric chloride only (group II), but rats in group IV showed nearly value toward the control group. Serum immunoglobulins (IgG) revealed no significant changes in serum immunoglobulins levels (IgG) between groups at 45 days. At 90 days group II showed significant decrease in IgG values compared to control, whereas both III and IV did not show significant changes compared to control group. Slight increase in serum lysozyme activity in group II and group III at 45 days, whereas rats in group IV did not show significant change compared with control rats. Histopatholog-
cal findings revealed alterations in group II and III different in severity and extension, while group IV showed mild alteration in different examined organs (livers, kidney and testes).

**INTRODUCTION**

Environmental pollution is a world wide problem. Heavy metals are belonging to the most important pollutants. Heavy metals constitute some of most hazardous substance that can bioaccumulate, a process in which a chemical pollutant enters into the body of animal and human and is low or not excreted but rather collected in their tissue (Ali and Amin, 2006).

Mercury is considered to be toxic to most organisms when present at appreciable levels. The total amount of mercury present in the environment as a result of natural erosion of the earth’s crust by wind and water far exceeds that caused by human activities. It disrupts most biological systems as a result of its affinity for sulfhydryl groups which are functional components of most enzymes and hormones (Hans et al., 1999).

Inorganic mercury is toxic to normal hepatic, neurological, reproductive, respiratory, immune, dermatological and developmental sequels (Risher and Amler, 2005).

Many pathological manifestations caused by exposure to mercury and other heavy metal have been reported in general, substantial evidence exists to support the hypothesis that mammalian immuno-compotent are sensitive to metals. Mercuric chloride is able to trigger immuno-dysregulation that leads either to auto immunity or immuno-suppression depending up on the genetic of the rat strain tested (Robert et al., 1995).

Recent evidences also show that mercury causes severe oxidative changes (Hansen et al., 2006), thus mercury proved to be a potential oxidant is the category of environmental factories. Therefore, there is a need to provide protection against mercury induced toxicity.

Historically, plants have been used as folk medicine against various type of diseases. Remedies from plants sources have proved to be very popular in primary health
care. Dates, the fruit of date palm (*Phoenix dactylifera*), represents an important highly nutritive source for human and animals. This has been attributed to constituents, of these constituents sugar, protein fat also contain some mineral Mg, iron, calcium, phosphorus, potassium and some vitamins as vit C, A, B complex, thiamine and nicotinic acid (AL-Shahhib and Marshall, 2003).

Some of properties of dates experimentally proved include antibiotic like effects on some bacteria (Abdel Salam, 1994), strengthens immune system (Puri et al., 2000).

So, the purpose of this experiment is to study the use of date Tamr as protective and preventive agent against mercuric chloride toxicity as well as to study some immunological, hematological and biochemical alterations and histopathological changes in rats intoxicated with mercuric chloride.

**MATERIALS AND METHODS**

**Dates:**

Dried dates were obtained from Egypt Assuit type and minced into small pieces for addition to ration

**Chemical:** Mercuric chloride was obtained from Merck Rohway, USA.

**Animals and experimental design.**

A total of 60 male albino rats weighing from 150 to 170 g were used. The rats were housed in plastic cages under good hygienic conditions and fed on balanced ration and water ad-libitum rats were divided into four equal groups:

- **Group I:** served as a control fed on a commercial basal diet.
- **Group II:** rats fed basal diet and administrated with 1/20 (LD50) mercuric chloride (2.29 mg/kg bw) in water. The lethal dose fifty of mercuric chloride was determined according to El-Boushy et al. (2000).
- **Group III:** rats fed on basal diet with 10% minced date as a prophylactic supplement 4 weeks before exposure to mercuric chloride (1/20 LD50) as group II.
- **Group IV:** rats fed basal diet supplemented with 10% minced dates 4 weeks before exposure of mercuric chloride (1/20 LD50) and both mercuric chloride and minced dates were continued for all experiment (90 days).

**Samples:**

Two blood samples were obtained from retro-orbital venous plexus at 45 and 90 days through the experiment.

Blood on EDTA anticoagulant solution for hematological profile. Blood in dry clean tube for collec-
tion of serum to estimate biochemical and hormone assay.

Blood on heparin anticoagulant solution (20 IU/ml blood) for measuring phagocytic activity of neutrophils

After sacrificed rats and post mortem examination, tissue specimens from liver, kidney and testis of animals groups were collected and preserved in 10% neutral buffered formalin at the end of the experiment.

Hematological examinations:

Hematological profile were carried out using automated cell counter, AL system, Germany (Feldman et al., 2000).

Serum biochemical analysis:

Estimation of biochemical and hormone assay included sodium and potassium which were determined according to Oser (1979). Total protein according to Sonnenwirth and Jarette (1980), albumin according to Drupt (1974), ALT and AST according to Reitman and Frankle (1957), urea according to Hovot (1985) and testosterone hormone according to Wilson and Foster (1992).

Serum immunoglobulins IgG:

Specific medial immunodiffusion plates (The binding site, BINDA RID, Birmingham (IgG) in sera mg/dl) according to Mancini et al. (1965).

Measurement of lysozyme activity:

Serum lysozyme activity was measured by agarose gel lyses assay according to the method described by Schltz (1987). Briefly, lyophate were prepared by dissolving 1% agarose in 0.06 M phosphate buffered saline at pH 6.3, 500 mg of Micrococcus lyso-deikticus in 5ml saline were added to 1 litre of agarose. Plates were poured then 25µl of serum samples and standard lysozyme were put in each well. After 18 hours the cleared zone diameter were measured to both standard lysozyme and serum samples and concentration was estimated.

Evaluation of phagocytic activity of neutrophils:

Phagocytosis of polymorphonuclear cells using Candida albicans was performed according to (Wilkinson, 1981), briefly, 5 ml heparinized blood were collected in plastic tube, added to 2 ml dextran 6% (MW, 500,000, Sigma) and leucocytes rich plasma was removed, suspended in Hank's balanced salt solution. In a plastic tube, 0.25 ml Hank’s solution, 0.25 ml pooled normal serum, 0.25 ml heat killed Candida albicans (5X 10^6/ml) and 0.25 ml leucocyte suspension (5 X 10^6 neutrophil/ml) for 30 minutes after which it was centrifuged. The supernatant was removed leaving a droplet into which the sediment was resus-
pended and slide smears were prepared and stained with Giemsa stain. Hundred neutrophils were examined for estimation of phagocytic percentage and index.

**Histopathological examination:**
Tissue specimens were previously fixed in 10% buffered formalin, processed for histopathological examination (Bancroft *et al.*, 1996).

**Statistical analysis:**
Data obtained in this experiment were statistically analyzed using analysis variance and comparing between groups were performed using least significant difference (LSD) at P<0.05 according to Petrie and Watson (1999) and computerized according to SPSS (2002).

**RESULTS**

**Hematological result:**
Tables (1 & 2) showed the changes in hematological values in rats treated with mercuric chloride. In group II and III (the same result but in less degree or values, respectively).

Mercuric chloride induced significant decreased in RBC count, Hb content and PCV percent, while red cell indexes (MCV, MCH and MCHC) did not show any significant variation (normocytic normochromic anemia). Significant decreased in WBC count (leucopenia). In the same tables show non significant changes in haemogram in group IV.

**Serum biochemical and hormone assay:**
Serum biochemical and hormone values of rats are illustrated in Tables (3 & 4). Group II showed significant increases in serum sodium, ALT, AST and urea, while serum potassium, total protein, albumin, globulin and testosterone were significant decreased.

Serum creatinine showed non significant changes at 45 days from beginning of the experiment but it significantly changes increased at 90 days. The same result were showed in group III but in less density, except creatinine showed non significant changes.

In group IV serum sodium, potassium, creatinine and testosterone revealed non significant changes. While serum ALT, AST, total protein, albumin, globulin and urea showed largely improved.

**Immunological findings:**
Phagocytic percentage and phagocytic index of neutrophil revealed significant decrease in rats received mercuric chloride only (group II) at 45 and 90 days compared with control rats (group I). Also, dates pre-treated animals with mercuric chloride (group III) showed significant decrease in phagocytic index at 45 days compared with control animals (Table, 5). While group IV pre and post -treated date with mercuric chloride
revealed non significant change compared to control.

**Serum immunoglobulins:**

Serum immunoglobulins IgG were illustrated in Table (6) and Fig. (1). There are no significant changes in serum immunoglobulins levels IgG between groups at 45 days, but at 90 days rats received mercuric chloride (group II) showed significant decrease in IgG value compared to control. Whereas both group II and group IV did not show significant changes compared to control group.

**Serum lysozyme activity:**

Both mercuric chloride intoxicated rats (group II) and dates pretreated animals with mercuric chloride (group III) exhibited significant increases in serum lysozyme activity at 45 days with respect to control (group I).

Whereas dates pre- and post-treated animals with mercuric chloride (group IV) did not show significant changes compared with control animals (Table, 7).

**Pathological findings:**

**Postmortem lesions:**

The post-mortem changes observed in liver, kidney and testis of sacrificed rats revealed enlargement, congestion and petechial haemorrhage. In groups III showed similar post mortem but less in number and severity. While no gross lesions were detected in rats of group IV.

**Histopathological findings:**

Histopathological examination was carried out on liver, kidney and testis revealed alteration in groups II and III. The alteration in the two groups were more or less similar but they different in severity and extension while group IV showed mild alteration in different examined organs.

In group II the livers showed sever reaction as necrosis and atrophy of hepatic cell. Hemorrhage and thrombosis formation in most hepatic vessels Fig (2). In the same group other rats liver showed focal necrosis with aggregation of few inflammatory cells (Fig, 3). Beside thrombosis and sever necrosis of hepatocytes, the liver showed granuloma formation consists of necrosis of liver cells and infiltrated with inflammatory cell (Fig, 4).

In group III microscopic examination of liver showed congestion of hepatic sinusoids, haemorrhage and atrophy of some hepatic cords (Fig, 5).

Moreover in group IV liver revealed mild hepatic necrosis with inflammatory cells infiltration (Fig, 6).

Regarding the kidney, the histopathological examination in group II revealed sever infiltration
of mononuclear inflammatory cells, as well as congestion of blood vessels accompanied by degeneration and necrosis of cells lining the renal tubules (Fig, 7 & 8).

Associated with degeneration and necrosis of cell lining renal tubules, the kidney showed formation of renal cast inside the lumen of renal tubules (Fig, 9). The cortical vasculature appeared diluted and engorged with blood (Fig, 10). Another alteration found sever formation of different size of thrombosis.

In group III histopathological examination of kidney showed moderate degree of congestion in renal blood vessels and atrophy of some glomeruli (Fig, 11). While group IV kidney showed mild degeneration of the lining tubular epithelium with few hyaline casts in some renal tubules (Fig, 12).

Microscopic examination of testicular tissue in group II revealed necrosis of spermatogenic cell layers (Fig, 13) with oedema and infiltration with mononuclear inflammatory cell in interstitial space. While in group III showed the same necrosis of spermatogenic cells beside congestion and interstitial oedema. In group IV showing nearly normal seminiferous tubules (Fig, 14).
Table (1): Mean values ± S.E. of hematogram of different experimental groups of rats at 45 days of the experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>RBCs x 10^6/ml</th>
<th>Hb g/dl</th>
<th>PCV %</th>
<th>MCV fl</th>
<th>MCH Pg</th>
<th>MCHC g/dl</th>
<th>WBCs x 10^3/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI</td>
<td>6.13 ± 0.151a</td>
<td>14.83 ± 0.216a</td>
<td>44.0 ± 0.706a</td>
<td>71.98 ± 2.381</td>
<td>24.23 ± 0.65</td>
<td>33.75 ± 0.686</td>
<td>8.00 ± 0.217a</td>
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<td>GII</td>
<td>5.0 ± 0.112b</td>
<td>12.33 ± 0.124c</td>
<td>35.0 ± 0.747c</td>
<td>70.64 ± 2.64</td>
<td>24.70 ± 0.82</td>
<td>35.66 ± 0.857</td>
<td>7.30 ± 0.194b</td>
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<td>GIII</td>
<td>5.4 ± 0.105b</td>
<td>13.26 ± 0.185b</td>
<td>38.33 ± 0.957b</td>
<td>70.98 ± 2.10</td>
<td>24.55 ± 0.73</td>
<td>35.00 ± 0.759</td>
<td>7.55 ± 0.215b</td>
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<td>GIV</td>
<td>6.0 ± 0.134a</td>
<td>14.56 ± 0.138a</td>
<td>42.33 ± 1.192a</td>
<td>70.27 ± 2.24</td>
<td>24.2 ± 0.71</td>
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<td>F-calculated</td>
<td>46.06#</td>
<td>47.16#</td>
<td>19.34#</td>
<td>1.321</td>
<td>1.245</td>
<td>1.329</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.008</td>
<td>0.002</td>
<td>0.300</td>
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</table>

# Significant at P < 0.05

a, b, c insignificant different between similar litter at P < 0.05
Table (2): Mean values ± S.E. of haematogram of different experimental groups of rats at 90 days of the experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RBCs x 10^6/ml</th>
<th>Hb g/dl</th>
<th>PCV %</th>
<th>MCV Fl</th>
<th>MCH Pg</th>
<th>MCHC g/dl</th>
<th>WBCs x 10^3/ml</th>
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</thead>
<tbody>
<tr>
<td>GI</td>
<td>5.86 ± 0.23a</td>
<td>15.0 ± 0.20a</td>
<td>46.3 ± 1.02a</td>
<td>79.07 ± 3.15</td>
<td>26.71 ± 0.89</td>
<td>32.66 ± 0.78</td>
<td>7.8 ± 0.27a</td>
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<tr>
<td>GII</td>
<td>3.8 ± 0.12c</td>
<td>10.16 ± 0.10c</td>
<td>30.33 ± 0.94c</td>
<td>80.14 ± 2.67</td>
<td>26.91 ± 0.87</td>
<td>33.48 ± 0.80</td>
<td>6.25 ± 0.19b</td>
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<tr>
<td>GIII</td>
<td>4.65 ± 0.10b</td>
<td>13.25 ± 0.21b</td>
<td>37.13 ± 0.80b</td>
<td>81.1 ± 2.51</td>
<td>28.5 ± 0.86</td>
<td>35.00 ± 1.10</td>
<td>7.5 ± 0.24a</td>
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<tr>
<td>GIV</td>
<td>5.5 ± 0.18a</td>
<td>14.6 ± 0.30a</td>
<td>44.0 ± 0.79a</td>
<td>80.12 ± 2.95</td>
<td>26.61 ± 0.70</td>
<td>33.2 ± 0.80</td>
<td>7.8 ± 0.33a</td>
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<tr>
<td>F-calculated</td>
<td>31.001#</td>
<td>106#</td>
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<td>0.001</td>
<td>0.137</td>
<td>0.050</td>
<td>0.007</td>
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# Significant at P < 0.05
a, b, c insignificant different between similar litter at P < 0.05
Table (3): Mean values ± S.E. of serum biochemical and hormone assay of different experimental groups of rats at 45 days of the experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Na mEq/l</th>
<th>K mEq/l</th>
<th>ALT u/l</th>
<th>AST u/l</th>
<th>T. Protein g/dl</th>
<th>Albumin g/dl</th>
<th>Globulin g/dl</th>
<th>A:G ratio</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
<th>Testosterone ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>149.3 ± 1.985&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.95 ± 0.091&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27 ± 0.593&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.8 ± 0.118&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.16 ± 0.093&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.86 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.68 ± 0.03</td>
<td>20.5 ± 0.635&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20 ± 0.042</td>
<td>2.55 ± 0.054&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>GII</td>
<td>174.0 ± 2.056&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.33 ± 0.101&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62 ± 1.922&lt;sup&gt;d&lt;/sup&gt;</td>
<td>79.6 ± 1.835&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.70 ± 0.151&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.10 ± 0.074&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.6 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.5 ± 1.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.50 ± 0.077</td>
<td>1.87 ± 0.033&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>156.0 ± 2.208&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.33 ± 0.159&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>3.33 ± 0.072&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.8 ± 0.13&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>47.0 ± 1.019&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>2.00 ± 0.062&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>GIV</td>
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<td>7.15 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37 ± 0.663&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.3 ± 1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.75 ± 0.158&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.52 ± 0.085&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.18 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.3 ± 0.824&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2.44 ± 0.016&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
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</table>

# Significant at P < 0.05
a, b, c, d insignificant different between similar litter at P < 0.05
Table (4): Mean values ± S.E. of serum biochemical and hormone assay of different experimental groups of rats at 90 days of the experiment.

<table>
<thead>
<tr>
<th>Parameter group</th>
<th>Na (mEq/l)</th>
<th>K (mEq/l)</th>
<th>ALT (u/l)</th>
<th>AST (u/l)</th>
<th>T. Protein (gm/dl)</th>
<th>Albumin (gm/dl)</th>
<th>Globulin (gm/dl)</th>
<th>A:G ratio</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>152 ± 2.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8 ± 0.089&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.4 ± 0.624&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38 ± 0.505&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5 ± 0.125&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.86 ± 0.093&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.66 ± 0.094&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.454 ± 0.025&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22 ± 0.682&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25 ± 0.044&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.55 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GII</td>
<td>188.3 ± 2.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.5 ± 0.072&lt;sup&gt;c&lt;/sup&gt;</td>
<td>105 ± 3.255&lt;sup&gt;d&lt;/sup&gt;</td>
<td>109.6 ± 2.526&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.8 ± 0.122&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.7 ± 0.065&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.6 ± 0.050&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.689 ± 0.019&lt;sup&gt;c&lt;/sup&gt;</td>
<td>94.66 ± 1.495&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.1 ± 0.038&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.29 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GIII</td>
<td>170 ± 1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.0 ± 0.151&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.3 ± 1.658&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.3 ± 1.173&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.85 ± 0.164&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.1 ± 0.067&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.8 ± 0.056&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.725 ± 0.030&lt;sup&gt;c&lt;/sup&gt;</td>
<td>64.33 ± 1.394&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.46 ± 0.075&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.70 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>GIV</td>
<td>158 ± 3.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0 ± 0.127&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.6 ± 1.154&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>5.60 ± 0.148&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.74 ± 0.079&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3 ± 0.075b</td>
<td>1.561 ± 0.031&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.22 ± 1.134&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 0.046&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.30 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F-calculated</td>
<td>34.2&lt;sup&gt;#&lt;/sup&gt;</td>
<td>99.4&lt;sup&gt;#&lt;/sup&gt;</td>
<td>309&lt;sup&gt;#&lt;/sup&gt;</td>
<td>333&lt;sup&gt;#&lt;/sup&gt;</td>
<td>62.2&lt;sup&gt;#&lt;/sup&gt;</td>
<td>39.52&lt;sup&gt;#&lt;/sup&gt;</td>
<td>42.79&lt;sup&gt;#&lt;/sup&gt;</td>
<td>21.9&lt;sup&gt;#&lt;/sup&gt;</td>
<td>62.7&lt;sup&gt;#&lt;/sup&gt;</td>
<td>22.5&lt;sup&gt;#&lt;/sup&gt;</td>
<td>39.12&lt;sup&gt;#&lt;/sup&gt;</td>
</tr>
<tr>
<td>Probability</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

# Significant at P < 0.05
a, b, c, d insignificant different between similar litter at P < 0.05
Table (5): Phagocytic percentage and index of rat neutrophils of different groups throughout the experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time</th>
<th>45 days</th>
<th></th>
<th>Index</th>
<th>90 days</th>
<th></th>
<th>Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td></td>
<td>Index</td>
<td></td>
<td>%</td>
<td></td>
<td>Index</td>
</tr>
<tr>
<td>Control I</td>
<td>51.0±2.0A</td>
<td>1.26±0.03A</td>
<td>52±3.9A</td>
<td>1.25±0.05A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxic group II</td>
<td>39.0±1.0</td>
<td>1.13±0.03aB</td>
<td>43±1.8a</td>
<td>1.10±0.03a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro-treated + Toxic III</td>
<td>43.0±4.0</td>
<td>1.14±0.89ac</td>
<td>45±2.8</td>
<td>1.10±0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated + Toxic IV</td>
<td>52.0±4.0b</td>
<td>1.25±0.08bc</td>
<td>15±1.3</td>
<td>1.20±0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-calculated</td>
<td>4.27</td>
<td>4.42</td>
<td>2.7</td>
<td>2.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (6): Serum immunoglobulins IgG (mg/ml) in sera of albino rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>45 days</th>
<th></th>
<th>90 days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>15.5 ± 0.4</td>
<td>16.2 ± 0.5A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>16.0 ± 0.6</td>
<td>14.0 ± 0.9ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>16.4 ± 1.2</td>
<td>17.7 ± 1.3b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>17.0 ± 0.3</td>
<td>17.6 ± 0.6b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-calculated</td>
<td>1.26</td>
<td>4.11*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (7): Serum lysozyme (µg/ml) of albino rats in different groups throughout the experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>45 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>463 ± 33A</td>
<td>547 ± 14</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>598 ± 24AB</td>
<td>607 ± 27A</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>579 ± 27ac</td>
<td>607 ± 29B</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>470 ± 34bc</td>
<td>523 ± 27ab</td>
<td></td>
</tr>
<tr>
<td>F calculated</td>
<td>5.85*</td>
<td>2.22*</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at P< 0.05 using ANOVA test, Bb, Cc and Dd are significantly different between two comparison groups against Capital letters using LSD at P<0.05.
Fig (1): Shows radial immunodiffusion IgG of examined rat (RID IgG)

Fig (2): Liver of rat showing necrosis and atrophy of hepatic cell & thrombus formation of most the hepatic vessels group II (H & E X 200).
Fig (3): Liver of rat showing focal area of necrosis with aggregation of few inflammatory cells group II (H& E X 400).
Fig (4): Liver of rat showing granuloma formation with necrosis of the hepatic cells and congestion of the hepatic sinusoid group II (H & E X400).

Fig (5): Liver of rat showing congestion and dilation of some hepatic sinusoids and atrophy of the hepatic cords group III (H & E X 100).

Fig (6): Liver of rat showing hepatic necrosis with inflammatory cells infiltration group IV (H & E X 400).
Fig (7 & 8): Kidney of rat showing severe infiltration of mononuclear inflammatory cells as well as degeneration and necrosis of cell lining the renal tubule group II. (H & E X 200).

Fig (9): Kidney of rat showing formation of renal cast inside the lumen of tubules accompanied by degeneration and necrosis of renal tubules group II. (H & E X 200).
Fig (10): Kidney of rat showing congestion and dilatation of some renal blood vessels. (H & E X 200).

Fig (11): Kidney of rat showing congestion of blood vessels, degeneration of some lining of epithelial tubules and atrophic glomeruli (H & E X 400).

Fig (12): Kidney of rat showing mild vascular degeneration of lining tubular epithelium with few hyaline casts in some renal tubules group IV (H & E X 100).
Fig (13): Testis of rat showing necrosis of the lining spermatogenic cell and degeneration of some interstitial cells group II (H & E X 200).

Fig (14): Testis of rat showing congestion and degeneration of some lining spermatogenic tubules, interstitial oedema and degeneration of some interstitial cells group III (H & E X 200)

Fig (15): Testis of rat showing nearly normal semineferous tubules Group IV (H & E x 200).
DISCUSSION

Heavy metal are considered as dangerous substances causing health hazards to human and animals through progressive irreversible accumulation in their bodies as a result of a repeated consumption of small amounts of these metals. Mercury is capable of damaging the organism in many ways due to its high affinity to various tissues and its tendency to accumulates (Radwanska et al., 1993).

In Egypt mercury in relatively source metal that enters the fresh water via industrial processes (Abd EL-Hamid, 1994). Date fruits are important and readily source and as a good source of nutritionally important mineral, vitamins, pholins, fibres, lipid and free from cholesterol (Mahmoud et al., 2003).

In our results mercuric chloride induced a type of normocytic normochromic anemia in group II and group III, with different in degree and accompanied by leucopenia. Many authors recorded anemia during mercuric chloride treatment as Lecavalier et al. (1994) and (Mohamed, 2004) who reported that there were normocytic normochromic anemia in rats and guinea pigs after administrated mercuric chloride in drinking water in different doses and duration. Normocytic normochromic anemia due to depression of erythrogenesis as in bone marrow hypoplasia as with poisoning (Bernard et al., 2000).

Leucopenia observed in our examination may be attributed to a number of metals that alter leucocytic count are known to markedly alter the equilibrium between the circulating and marginated compartments. It produce apparent leucopenia by increasing the size of the marginated pool (Wand and Colin, 1998).

These changes in haemogram may be attributed to affect of heavy metal directly on blood cell and indirectly on haemopiotic organs.

In our study the significant increase in serum sodium and decreases in serum potassium levels in groups II and III may be due to the gradual development of renal function impairment as well as tubular damage (EL-Boushy et al. 2000), these alteration in sodium and potassium mainly attributed to the toxic effect of mercuric chloride which seriously affect the cell membrane function leading to disturbance of the ion regulation.

The elevation in serum ALT and AST activity, the liver is consider as a second target depot for mercury in terms of total amount irrespective of administration routs. These elevation of the these enzymes attributed to severs dam-
age in liver cells which releasing the hepatic enzyme into serum (Ibrahim et al., 1991) and (Ga-
mal, 1991) these finding was con-
frmed histopathologically by de-
genration and necrosis of liver
cell.

The decrease in serum total proteins and albumin and globulin may be attributed to decrease in protein synthesis by hepatic cell and increase rate of excretion due to renal damage. Our results are similar to result recorded by EL-
Boushy et al. (2000).

Significant increase of serum urea and creatinine in our result was indicating marked the nephro-
toxic effect observed after expo-
sure of inorganic mercury is as-
sumed to be related to the fact that the kidney accumulates more mer-
curry than other organ in the body (Osfar and Ibrahim, 2000 and Mohamed, 2004).

The observed reduction in se-
rum testosterone level agree with the result of EL-Boushy et al. (2000). The decreased of testoster-
one may be as result of direct dam-
age of mercuric chloride in leydig cells (Robert et al., 1995).

The present investigation re-
vealed that groups II received mer-
curic chloride only intoxication causes significant decrease in phagocytic percentage and index of neutrophils compared to control animals or groups IV. The principle toxic effects of mercury involve interactions with large num-
ber of cellular processes, including the formation of complexes with free thiols and protein thiol groups, which may lead to oxidative stress (Stacey and Kappus, 1982).

Our results agree with Soltys et al. (1997) who found that sub-
chronic exposure to mercury to guinea pigs resulted in significant decrease in phagocytic activity of peritoneal macrophages through the experiment. Also, Worth et al. (2001) recorded mercuric chloride inhibited polarization and immu-
noglobulin-mediated phagocytosis in a dose-dependant manner in hu-
man neutrophils. Moreover, Hem-
dan et al. (2007) found that expo-
sure to mercuric chloride signifi-
cantly reduced peripheral blood mononuclear cell vitality in vitro as well as elicited distinct effects on T-helper 1 (Th1) and T-helper 2 (Th2) cytokine expression depending on cellular activation path-
ways. Furthermore, Naz et al. (2008) found that subchronic ex-
posure with mercuric chloride in rats results a decrease of 33% im-
mune response as evident from antinuclear antibody (ANA) positive test.

The present results in group IV; toxicated with mercuric chlor-
ride and treated with dates till the end of experiment showed nearly
values of phagocytic activity of neutrophils compared to control group that means ameliorative effect of dates on adverse effect of mercuric chloride. Dates contain carbohydrate, fat, protein, vitamins, fiber and at least 15 minerals, including selenium which play an important role in immune function and prevent cancer (AL-Shahhib and Marshall 2003). Dates possess strong antioxidant activity which provokes free radical scavenging enzyme system. Antioxidants are compound that can delay or inhibit oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction (Velioglu et al., 1998).

These results indicating significant immunomodulating effect of dates, which agree with the results of Puri et al. (2000) who recorded an enhancement of macrophage activation and increased in haemagglutination antibody titres as well as plaque forming cell (PFC) counts in mice orally administrated date extract. The immunostimulatory activity of dates may be attributed to presence of β-D-glucan in date flesh. Ishurd et al. (2002) isolated β-D-glucan from the fruit of dates. β- D-glucan is an important candidate molecule of biological response modifier implicated in cancer, it can be obtained from other sources such as yeast, fungi, bacteria and plants. Tokunako et al. (2000) mentioned that β- D-glucan has higher biological effect in activation of alternative pathway of complement, induce interleukin-6 synthesis of macrophage in vitro, and act as adjuvant effect on antibody production.

Our results indicate that ameliorative effect of dates on mercuric chloride toxicity. Host resistance is highly dependent on the first line of defence, phagocytes; there is not necessarily a direct correlation between serum immunoglobulin levels and host resistance (Robert et al., 1995). Our data exhibited significant decrease of IgG at 90 day in mercuric chloride toxicity (group II) compared to control. The present results agree with Shenker et al. (1993) who reported immunototic effect of mercuric chloride on human lymphocytes and monocytes; they suggest that mercuric chloride at low and high dose inhibit activation of human B cells to synthesize IgM and IgG. Contradiction of this result, Lawreuce et al. (1983) who found that low dose of mercuric chloride enhance IgM production.

Bagenstose et al. (2001) and Zheng et al. (2005) reported that subtoxic doses of mercuric chloride in SJL mice resulting in increased production of serum immunoglobulin IgE and IgG1, interleukin 4 and enhances the suscepti-
bility to Leishmaniasis.

Lysozyme are proteins of low molecular weight found in polymorphonuclear leukocytes and mononuclear cells, lysozyme are considered as a member of innate humoral factors that elaborate from the body and showed dramatic increase in concentration in response to infection or tissue injury (Weir, 1983). Lysozyme in serum can destroy the glucosidic bond in cell wall of *Escherichia coli* and *Staphylococcus* as a result of phagocytic activity (Guo et al., 2004). Our data revealed increased lysozyme activity in mercuric chloride group II and group III at 45 days. This increase may be attributed to activation effect of mercuric chloride on immune cells or may be due to tissue injury as a result of harmful effect of mercuric chloride. Zheng et al (2005) reported elevated production of B cell activating factor in susceptible mice administrated mercuric chloride.

The histopathological lesions appeared approximately the same in group II, III and IV but differ in severity and extention.

The lesions observed in liver, the central vein and blood sinusoid were dilated and engorged with blood, some times considerable areas of haemorrhage were observed. These changes were attributed to toxic effect of mercuric chloride on the endothelial cells lining blood vessels [Hans et al. (1999) and Mohamed (2004)]. Several type of degenerative changes in hepatocytes and necrosis was observed which local or diffused in its distribution. These changes occurred most commonly due to the direct toxic effect of heavy metals on hepatocytes through the blood stream.

Kidney lesions were demonstrated by blood vessels were congested and haemorrhage. These lesions attributed to direct toxic effect of mercury on development and function of endothelial lining of renal blood vessels. A toxic tubular nephritis were observed as well as the epithelial lining of the renal tubules appear degenerated and some tubules contain hyaline cast these result agree with Hamza et al. (1994); Carlton and McGowin (1995).

The testis showed different alteration, these alteration manifested by different degree of degeneration and or necrosis in the lining spermatogenic tubules, interstitial oedema, congestion and infiltration of inflammatory cell. The lesions in the testis attributed to effect of mercuric chloride on endothelial cells of small vessels leading to its damage which give rise to increased capillary permeability, this lead to vascular escape of fluid and blood plasma substance into interstitial which re-
sults oedema, decrease capillary blood flow ischemia and testicular degeneration (Robert et al., 1995) and EL-Boushy, et al. (2000).

From our result mention before we found that the two groups treated with date (III and IV) have variation if compared with group treated mercuric only II. These variation due to date supplemented and his effect on toxicity of mercuric. In group III (pretreated with date) the values of hemogram, biochemical and alteration of histopathological are less degree of severity than group II (moderate reaction), where the date improved the feed utilization and healthy condition of animal and act as immunostimulant (Ali et al., 1999). This improvement play role against mercury toxicity. By time this improvement was decreased by stopped supplementation of date so must be continuous supply of date through experiment time to avoid hazardous effect of date, these what done in group IV.

The improvement of hemogram, biochemical and histopathological studies which showed in results of group IV due to the effect of date on mercuric toxicity which play role to prevent the hazardous effect of mercury.

Authors discussed the quality and nutritive value of date are influence by their chemical composition. They recorded that, the dates contain essential nutrient, high sugar and moderates amount of protein and lipid. Also, they contain minerals (iron, manganese, copper, zinc, cobalt, sodium potassium, phosphorus, calcium and choline; and vitamins A, C, B complex (Al-khouli et al., 1998; EL-Ghazoli and Hussin, 2003; and Mahmoud et al., 2003). Moreover, the date contain antioxidant, vitamins and elements that play major role in prevention of heavy metals toxicity (Sharma et al., 2007 and Youseif et al., 2007).

The date contain vitamin C, which has strong antioxidant, having nuclophilic properties and binding to mercury ions to reduced mercury damage effect (Sato et al., 1997). EL-Sharkawy and EL-Nesr (2008) reported that, supplementation of vitamin C to mercuric chloride treated rabbits has effectively increase antioxidant enzymes (glutathione peroxidase and superoxide dismutase) and reduced histopathological lesions. Moreover Luck et al. (1995) found that ascorbic acid effects both integrity of tubular structure and functionality of the testes.

The dates contain vitamin A which has antioxidant effect as antioxidant substance degrade free radical damage proteins and remove lipid hydroperoxide from membrane (Diplock, 1995). Vitamin A increase the immune response and or modulate the re-
sponse into the desired direction and thus may improve and prevent any cellular damage caused by oxygen radicals through the antioxidant effect of vitamin (Zaki and Zaki, 2006).

Date contain zinc and iron, they play important role in reducing nephrotoxic effect of mercury and prevent lipid peroxidation, respectively (Robert et al., 1995 and Silver and Waldan, 1997). Additionally, dates possess copper and cobalt which are promoting hematopoiesis (Yourgreatpet, 2004).

The constituents of dates improve the general healthy condition of animal and can reduce the adverse effect of mercuric chloride toxicity. Dates today considered as food function (Ali et al., 1999).

It was concluded that addition of dates to ration reduce adverse effect of mercuric chloride toxicity on hematological, biochemical, some immunological and pathological changes in rats.

REFERENCES
Bernard, F.; Joseph, G and


وقد تم إجراء التجربة على ستين فأرا مقسمة إلى أربعة مجموعات متساوية، المجموعة الأولى وهي الضابطة أما الثانية تم اعطاؤها 10% من الجرعة النصف الميانت من كلويرد الزئبق والثالثة تم تغذيتها بالعنيدة الأساسية مضافًا إليها 10% من التمر المجروح قبل تعرضها إلى كلويرد الزئبق والمجموعة الرابعة تم تغذيتها على العلامة الدراسية مضافًا إليها 10% من التمر المجروح لمدة 4 أسابيع قبل التعرض إلى كلويرد الزئبق واستمر اعطاء التمر حتى نهاية التجربة.

أما بالنسبة إلى النتائج صور الدم الكامل في المجموعة الثانية والثالثة وجد انيميا طبيعية الحجم واللون بالإضافة إلى نقص ملحوظ في عدد كرات الدم البيضاء ولكن لا توجد أي تغيرات معنوية في صورة الدم للمجموعة الرابعة. وجد قياس الصوديوم وانزيمات الكبد والبوليون في مصل الفرامل، وجدت زيادة معنوية في المجموعة الثانية بينما وجد نقص في قياسات كل من البولاسيم والإيبوترين والجلوبيولين وهورمون التستوستيرون في نفس المجموعة وقد زادت نسبة الكرياتينين في اليوم التاسع.

وقد أظهرت المجموعة الثالثة نفس النتائج السابقة ولكن بدرجة أقل ما عدا قياس الكرياتينين الذي لم يظهر أي تغير معنوي أما بالنسبة للمجموعة الرابعة لا يوجد تغير معنوي في النتائج الصوديوم البولاسيوم والكرياتينين وهورمون التستوستيرون. ولكن بالنسبة لقياسات إنزيمات الكبد والبوليون والبوليوبين والبوليوبين والبوليون.

فوق وجد أنها تحسن كثيرا في هذه المجموعة.

وقد وجد أن كلويرد الزئبق يؤدي إلى انخفاض نسبة وعامل الكفاءة البلعومية لخلايا البتروفيل في المجموعة الثانية ولكن بالنسبة للمجموعة الرابعة فقد اظهرت النتائج فيما تتجه ناحية المجموعة الضابطة. وقد تم إجراء الاختبار الكمي للجلوبيولينات المناعية (Immunoglobulin IgG) في قياسات آل IgG في قياسات آل IgG في المجموعة الثانية للمقارنة بالمجموعة الضابطة بينما لم تظهر المجموعة الثالثة والرابعة أي تغير معنوي بالمقارنة بالمجموعة الضابطة.

وقد وجدت زيادة كبيرة في قياس نشاط الليزوزيم في المجموعة الثانية والثالثة في اليوم الخامس والأربعون بينما لم تظهر أي تغيرات في المجموعة الرابعة بالمقارنة بالمجموعة الضابطة.

وعند الفحص البصري للكبد الكلي والخصية فقد وجد احترافات في الأنسجة المختلفة لهذه الأعضاء في كل من المجموعة الثانية والثالثة مع الاختلاف في شدتها وانتشارها بينما أظهرت المجموعة الرابعة تغيرات بانولوجية طفيفة.

المحكمون:

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