Renal effects of propolis and malic acid in Aluminium Exposed Male Rats.

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Abstract

The comparative effect of propolis and malic acid in reducing the effects of oral Aluminium chloride administration on kidney functions of adult male rats were studied. Forty adult male rats were divided randomly into four equal groups; first group was a control group (C group), animals of 2nd group received AlCl3 (50 mg/kg body weight) (Al group), animals of 3rd group received AlCl3 (50 mg/kg body weight) + propolis (50 mg/kg body weight) (AL group); the animals of the 4th group administered with AlCl3 (50 mg/kg body weight) + Malic acid (45 mg/kg body weight) (ALM group). The experiment lasted for 60 days rats of different groups received the above materials through a stomach tube. Results of the present study showed that kidney function parameters were significantly elevated by AlCl3 administration for 60 days. The combination of AlCl3 plus propolis or malic acid reduced this significant elevation to a semi normal values. On the other hand examination of kidney H&E stained sections showed the negative and deleterious effects of Aluminium, these effects were improved in rats received propolis or malic acid.

Key words: Aluminium chloride, propolis, malic acid, kidney function.

Introduction

Various environmental chemicals, industrial pollutants and food additives have been implicated as causing harmful effects. Aluminium (Al), the third most common element approximately 8% of total mineral components in the earth’s crust found combination with oxygen, silicon, fluorine and other elements in the soil, rocks, clays and gems (Greenwood and Earnshaw, 1997). Al is widely distributed in the environment and extensively used in daily life, which causes its easy exposure to human beings. It gets access to the human and animals body via the gastrointestinal and the respiratory tracts (Domingo et al., 1993). Al accumulates in all tissues of the mammals, and has a significant toxic potential for humans and animal’s tissues mainly due to its deleterious effects on lipid peroxidation and induction of oxidative stress (Mohammadirad et al., 2011). Aluminium provoked and neurotoxicity (Stojanovic and Ninkovic, 2009), hematotoxicity (Turgut et al., 2007; Al-Qayim, 2013b) hepatotoxicity (Al-Qayim and Saadoon, 2013) nephrotoxicity (Shelley, 2012), cardiotoxicity (Greenwood et al., 1997) Besides, Al caused genetic damage in rat bone marrow cells (Balasubramanyam et al., 2009).

Propolis, a resinous wax-like beehive product is collected by honey bees from plant exudates and also known as bee glue. The worker bees apply the resin to seal any cracks and fissures in the hive and they”line their front door” with it to prevent contamination. They use it as an antiseptic in breeder cells, and they mix propolis with wax to distribute a fine varnish over every inch of the hive to protect it. Chemical properties of propolis are not only beneficial to bees but have general pharmacological value as a natural mixture (Orsolic, 2010). Several empirical and clinical findings point to the fact that propolis may be more effective against pathogenic microorganisms and environmental pollutes like Lead (Al-Qayim et al., 2013a). The propolis has been used in folk medicine for antioxidant, immune-stimulating, anti-inflammatory and non-toxic natures (Cole et al., 2010; Hendi et al., 2011) Propolis has also been found to have powerful anti-inflammatory properties. Propolis can counteract the damaging effects of aluminium (Mahmoud et al., 2013).

Malic Acid has good chelating properties, chelating agents defined as a “chemicals that form soluble, complex molecules with certain metal ions such as aluminium, cadmium, lead, mercury, arsenic, inactivating the ions so that they cannot normally react with other elements or ions to produce precipitates or scale (Lin-Tong et al., 2012) Malic Acid is one of the most potent chelators of aluminium and was the most effective of several chelators tested at reducing aluminium overload (Charles et al., 1986; Rim, 2007; Crisponi et al., 2011). Treatment with Malic Acid has been shown to greatly increase the fecal and urinary excretion of aluminium and reduce the concentration of aluminium found in various organs and tissues (Barbier et al., 2005).
The aim of the present study was to evaluate the possible ameliorative role of propolis or malic acid against negative effects of aluminium chloride exposure could have on the functions of kidney of Wister rats.

**Material and methods**

**Experimental Animals**

A total of forty male Albino Wister rats were at age 8 – 9 weeks and their body weight ranged between 100 – 120 grams, were kept in the same suitable environmental conditions of 22 – 27°C, and photoperiod of 12 hours daily. After two weeks of adaptation, rats were divided randomly into four groups and each group contained ten animals;

- Group (C) : Control group orally administered distil water daily.
- Group (AL) : Aluminium group orally administered AlCl3 (50 mg /kg body weight), dissolved in distil water daily.
- Group (ALP) : Aluminium + Propolis group orally administered AlCl3 (50 mg /kg body weight) dissolved in distil water and propolis (50 mg /kg body weight) daily.
- Group (ALM) : Aluminium + Malic group orally administered AlCl3 (50 mg /kg body weight) dissolved in distil water and Malic acid (45 mg /kg body weight) dissolved in distil water. All applications were administered daily for 60 consecutive days. At The end of the experiment blood sample ( 4-5 ml ) was collected from the rat obtained via cardiac puncture technique from each anesthetized animal using disposable syringe (5 ml) and blood was withdrawn into plastic test tubes ( gel tube ) for serum isolation for biochemical analysis.

**Biochemical analysis**

Serum Creatinin estimated according to Jaffe;s reaction with picric acid in alkaline solution using commercial kit from RANDOX company. Total serum protein by biuret reaction using a commercial kit from Human Diagnostic Worldwide. Plasma urea determined by enzymatic method using a commercial kit from BIOMEREUX. Plasma Uric acid Uricase method using a commercial kit from BIOLABO.

**Histopathological changes**

Kidney tissues preserved in 10% neutral formalin buffer solution, after fixation, the tissue was trimmed and the specimen were washed with saline for (1-2hrs) and transferred to following steps:1. Dehydration: Specimen were passed through ascending grades of ethanol alcohol (70%, 80%, 90%, 100%). For 1 hour in each concentration.2. Clearing: Two solutions of xylol commonly used for clearing. The specimens rested 1 hour in each step.3. Impregnation with Paraffin wax. 4. Blocking. 5. Sectioning. 6. Staining with hematoxilin &eiosin.

**Statistical analysis**

Data are shown as the Mean± SE. Data were analyzed by using one way analysis of variance (ANOVA) within SPSS program. Means were tested by t test at probability level of (p<0.05).

**Results and discussion**

The ameliorative role of propolis and malic acid in kidney function parameters of Aluminium chloride exposed male rats for 60 days represented in table-1. The results of the present experiment showed that Aluminium chloride caused significant increase (p<0.0001) in level of creatinin, urea and uric acid (0.65±0.03, 0.42±0.04, 3.42±0.46) when compared with control group (0.2±0.01, 0.11±0.004, 1.48±17), this increase was modified significantly in the group received Aluminium chloride +malic acid (0.74±0.28,0.29±0.04,3.15±0.27) and in the group which received Aluminium chloride +propolis (0.74±0.28,0.32±0.036,2.74±0.38) to asemi normal values respectively, while there was anon significant increase (p<0.0001) in the level of total protein and albumin as compared with control group.

The role of Aluminium in causing kidney damage is receiving considerable attention, because the kidney is the first target organ of heavy metal toxicity because of its ability to reabsorb and accumulate diverval metals. The extent of renal damage by heavy metals depends on the nature, the dose, route and duration of exposure. Aluminium is excreted mainly by kidney in accordance, it has been reported that both acute and chronic intoxication cause nephropathies (19,8)

Laboratory evaluation of rodents kidneys is the same as that for domestic animals, and it involved evaluation of blood parameters, such as urea nitrogen, creatinin. Present data reveled that AlCl3 administration caused increase of serum urea and creatinine concentration. Increased in plasma urea nitrogen and creatinine concentrations was compromise about 75% of renal function. Chronic exposure to Aluminium also results disruptions in mineral balance disturbances. In the biological systems Aluminium ions replace iron and magnesium ions (Ward, 2001). They also alter cellular membrane structures and activity of many enzymatic processes, reduce Fe2+ binding to ferritin, and disturb hem synthesis (Abreo et al., 1993). Free iron ions released from biological complexes by Aluminium can catalyze hydrogen peroxide decomposition to hydroxyl radical- according to Fenton’s reaction. This high hydroxyl radical reactivity is able to initiate cellular damage (Bartosz, 1995; Forman et al., 1982)). So results of the present experiment denoted the deleterious effects of AlCl3 on renal functions in clearance of plasma which was demonstrated by elevation of plasma uric acid, urea, and creatinin. The present results indicated that administration of AlCl3 for 60 days consequently caused critical accumulation of this metal in kidneys leading to renal failure development. On the other hand the present result revealed that oral administration of
propolis or malic acid to animals exposed to AlCl3 revealed an ameliorative role in preventing renal failure as shown by significant reduction in renal function parameters such as serum creatinin, urea and uric acid concentration as a compared to group that received AlCl3 indicating an ameliorative role. It has been found that administration of propolis decreased lipid peroxidation of cellular membrane so it can play a prevention role against the free radical reaction (Sun et al., 2000; Newairy et al., 2009).

The chelating & antioxidant properties of malic acid has been well documented precisely (Elmenoufy, 2012; Lin-Tong et al., 2012). There for administration of malic acid protect kidney tissue from deleterious effect of AlCl3 (Domingo et al., 1993). Accordingly, the antioxidant activity of both propolis and malic acid and chelating activity of malic acid restore cellular integrity with maintaining kidney function including maintaining the secretion, reabsorption, and excretion of creatinin, urea and uric acid and consencountly regulate their serum concentration. The present results are in agree with (Crisponi et al., 2011) who found that honey (antioxidant) exhibit a protective potential by improving the disturbed kidney biochemical marker, that all alleviating the increase lipid peroxidation induce by Aluminium chloride, in addition effect is due to ability counteract the oxidative damage and protect kidney tissue and restore the normal metabolic process. On the other hand malic acid is known for its ability to increase energy (ATP) because it is an essential components in Krebs cycle and that (malic acid )consider as antioxidant (Crisponi et al., 2011). Thus, treatment with propolis or malic acid could improve cellular membrane & organ functioning more profoundly and brought all these variables in kidney function parameters concentration to ward control group. Therefore, both compounds can alleviate the Al-mediated kidney injury in rats.

The results of the present study have shown that light microscopic examination of kidney tissues sections revealed the negative and deleterious effects had with Aluminium chloride. Kidney tissue section of control group showed normal histological structure (figure-1), while in the second group which received Aluminium chloride (figure-2) showed Infiltration of inflammatory cells, congestion of blood vessels. In spite of the material deposed in the lumen of tubules, there was enlargement of epithelial cells lining urinary tubules leading to occlusion of some urinary tubules and stenosis in other. Other sections showed necrosis in other epithelial lining urinary tubules .In addition to parenchyma of the kidney. Furthermore, there were enlargement and vaculation of glomerular tuft. While in group that received Aluminium chloride + propolis showed milled congestion of blood vessels ,slight infiltration of mononuclear cells around blood vessels . With slight desquamation of epithelial lining the tubules, vaculation and necrotizing of epithelial lining tubules (figure-3), kidney tissue section of rats received Aluminium +malic acid showed milled congestion of blood vessels , slight infiltration of inflammatory cells , thickening of blood vessels wall. (figure-4). It is possible that the architectural derangement of kidney tissue observed in the present study may be due to altered cellular organelles like mitochondria, endoplasmic reticulum, lysosomes and cell membrane by aluminium. Aluminium chloride ip injection caused vacuolization of cellular organelles leading to structural and biochemical changes at sub cellular level (Saleh et al., 2013), other suggestion for the deleterious effects of Aluminium on kidney tissue made by (Kutlubay et al., 2007; Shilpi et al., 2009) who found in vitro study that Aluminium accelerates iron-induced lipid peroxidation .In general Aluminium was able to induce oxidative stress in many tissues (Al Kahtani, 2010). The denoted renal histopathological changes in the present study in regard to the protective effects of propolis against AlCl3 effects could be attributed to the potential anti-oxidant like effects of propolis( pdf)ag). Similirre results were cited by (Lote, 1995), reported that propolis modulated the immune/inflammatory response. In brief, propolis protected the body from the biohazards in immune-modulatory findings induced by gamma irradiated Nigella sativa. Similier results were cited by (Saleh et al., 2013) how found that showed disappearance of the lymphocytic infiltrate of the kidney of rats given Gamma Irradiated Nigella Sativa and Propolis (GRNSP), infiltration and desquamation in the epithelium of the renal tubules. The antioxidant properties of propolis prevent . The semi control features of kidney tissue of rats received malic acid with Aluminium chloride may be explained by the potential beneficial effects of malic acid as anti oxidant (Kabouj, 2007; Crisponi et al., 2011).

In conclusion the present study clarified that administration of propolis and malic acid are beneficial to prevent alterations induced by Aluminium chloride. Moreover, propolis and malic acid modulates changes in kidney function parameters and histopathology of kidney probably through they anti-oxidative action and its detoxification process as well as the potential to minimize the deleterious effects of free radicals on tissue.

Table1. Assessment of the ameliorative role of propolis and malic acid serum level of kidney function parameters Creatinin Total protein, albumin, urea, uric acid

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Creatinin</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Urea</th>
<th>Uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control *</td>
<td>0.21±0.01</td>
<td>5.58±5.77</td>
<td>10.94±0.93</td>
<td>0.11±0.004</td>
<td>1.48±17</td>
</tr>
<tr>
<td>AL *</td>
<td>0.97±0.03</td>
<td>5.58±0.35</td>
<td>9.23±0.87</td>
<td>0.42±0.04</td>
<td>3.42±0.46</td>
</tr>
<tr>
<td>AL+P *</td>
<td>0.74±0.28</td>
<td>5.49±0.12</td>
<td>9.99±0.77</td>
<td>0.32±0.036</td>
<td>2.74±0.38</td>
</tr>
<tr>
<td>AL+M *</td>
<td>0.65±0.28</td>
<td>4.99±0.14</td>
<td>11.57±0.87</td>
<td>0.29±0.04</td>
<td>2.15±0.27</td>
</tr>
</tbody>
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Small superscript denote significant (p<0.05) difference between groups (Column). * Orally administration
Figure 1. Histological section in the kidney of rat from control group. (X400, H&E stain)

Figure 2. Light microscopic image of rat kidney received AlCl3, show severe dilation with cystic appearance of renal tubules that lined with flake epithelial cells, congestion of blood vessels. Pretentious material deposited in the lumen of tubules associated with tubular necrosis in others as well as infiltration of inflammatory cells in kidney parenchyma, in addition to hypercellularity of glomerular tuft and consequently enlarged. (X400, H&E stain)

Figure 3. Light microscopic image of rat kidney received AlCl3 + Propolis. showed Congestion of blood vessels, slight infiltration of mononuclear cells around blood, present of hyaline cost, vaculation and slight necrotizing of epithelial lining tubules, vessels, and enlargement of glomerular tuft. X400, H&E stain

Figure 4. Light microscopic image of rat kidney received AlCl3 + Malic acid, show Congestion of blood vessels with fibromuscular hypertrophy, slight infiltration of inflammatory cells between tubules. X400, H&E stain
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References