The protective effect of Crataegus monogyna Jacq aqueous extract (fruits and leaves) on blood cells and lipid profile of rats after copper induced-toxicity

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ABSTRACT

The objective of this work is to use the Hawthorn Crataegus monogyna, as a protective agent against copper chronic intoxication. Male Wistar rats were divided into six groups; the control received tap water, standard diet ad libitum, two positive controls treated respectively with Hawthorn leaves and fruits aqueous extract, a group treated with Cu and finally, two groups treated with Cu+leaves (CuL) and Cu+fruits (CuF). The treatment was done by gavage for 30 consecutive days, where: glucose-6-phosphate of erythrocytes (G6PD), white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (HGB), hematocrits (HCT), mean corpuscular volume (MCV), triglycerides (TRIG), cholesterol (CHOL), high density lipoproteins (HDL) and low density lipoproteins (LDL) were measured. Copper treatment reduced G6PD, RBC, HGB, HCT, MCV, TRIG and CHOL levels, compared to the control. Compared to the Cu group, the two combined treatments (Cu L and Cu F) have an increase on G6PDH, RBC, HGB, HCT, MCV, TRIG and CHOL levels, with a decrease in WBC, PLT, and LDL levels. As a conclusion, hawthorn aqueous extracts have mitigated copper toxicity towards blood cells and LDL of wistar rats.

Keywords : Glucose-6-Phosphate Dehydrogenase, hawthorn, high density lipoprotein, red blood cells

INTRODUCTION

Copper is a trace element essential for many biological processes, but it becomes harmful when it exceeds the threshold level (Abbas et al., 2018). About 60% of consumed copper is absorbed in the stomach and the small intestine (DES, 2013), where its absorption, distribution, detoxification and elimination are well controlled (Kumar et al., 2015). Copper homeostasis maintains of copper distribution and prevents causing any negative effects to cellular defense system (Quamar et al., 2019). However, both augmentation and deficiency of copper concentration may cause physiological disorders (Chambers et al., 2010). Thus, increases in copper concentration in body have been reported to be associated with many pathological conditions (Parmar et al., 2002; Ozcelik and Uzun, 2009) as anemia by the red blood destruction (DES, 2013), abnormal lipid profile (Burkhead and Lutsenko, 2013) and lower triglycerides concentrations (Wuolikainen et al., 2014). Furthermore, high copper level provokes cell injury (Saravu et al., 2007) by oxidizing cell membranes (James et al., 1999; Saravu et al., 2007), mitochondrial dysfunction and lowering antioxidant enzymes, leading to oxidative stress damage (Tiwari et al., 2018).

Through the years, interest of using plant compounds has been growing faster in worldwide due to their benefits on health (Nandi and Ghosh, 2016). Hawthorn, Crataegus monogyna, is one of very common shrub plant used in medicinal treatments (Fong and Bauman, 2002), which considered a relatively safe herb and without serious adverse effects (Zapfe, 2001). The plant is well distributed in the Mediterranean region. C. monogyna is rich in proanthocyanidins and flavonoids (Bahorun et al., 1996), which are superoxide anion (Keser et al., 2014), hydroxyl radical, hydrogen peroxides scavengers and lipid peroxidase reducer (Bahorun et al., 1994; Rice-Evans, 2004), which make it a powerful antioxidant (Yao et al., 2008). Interestingly, flavonoids of Hawthorn have the ability to inhibit copper intake (Kuo et al., 1998).

The aim of this study is to investigate the ability of the common C. monogyna aqueous extract of both fruits and leaves in protecting blood
biomarkers and lipid profile of Wistar rat intoxicated with copper sulfate.

**MATERIALS AND METHODS**

**Plant and preparation**

*Crataegus monogyna* is grown spontaneously along the Algeria northern zone, exceeding 3 meters in length, and characterized by green leaves, white flowers and red fruits; the latter reaches maturity in mid-autumn. Fruits and leaves were harvested freshly in November from Annaba area, northeastern Algeria. 1.5g/kg bwe of fruits (F) and leaves (L) were weighted daily, crushed in an appropriate volume of distilled water (where each rat takes 1ml of the obtained extract) and were kept overnight at room temperature. The two homogenates were filtered in the morning for obtaining the of F and L aqueous extract. Copper sulfate powder was dissolved daily directly before carrying out the tests in distilled water. The mixture of copper + F and copper + L where prepared daily using the same doses.

**Experimental design**

Wistar rats were purchased from the Pasteur institute, Algiers (Algeria) weighing 196±8 g, that received tap water and standard diet *ad libitum*. Thirty-six males were divided equally into 6 groups; the control (C) having a standard diet, the copper (Cu: 100 mg CuSO₄·5H₂O/Kg bw), the fruits (F:1.5 g fruits/kg bw), the leaves (L: 1.5 g leaves/kg bw), the Cu+ F (100 mg CuSO₄·5H₂O /Kg bw + 1.5 g fruits/kg bw) and the Cu+ L (100 mg CuSO₄·5H₂O /Kg bw + 1.5 g leaves/kg bw) group. Rats were sacrificed by decapitation after 30 consecutive days of copper oral administration, fruits and leaves solutions. Blood was collected in heparinized and EDTA test tubes, in which heparinized tubes were centrifuged at 3000 rpm for 10 minutes, and then the plasma was stored at -20°C till further analysis. Animals’ experiments were authorized by the Ethical Committee of Animal Sciences at the University at the Badji Mokhtar university of Annaba (Algeria).

**Erythrocytes G6PD assay**

Glucose-6-phosphate dehydrogenase (G6PD) dosage was measured using Mindray BS-380 apparatus, according to BIOLABO REAGENT (U.V Kinetic method) kit and the reaction scheme (Beutler et al., 1977). The rate of increase in NADPH concentration measured at 340 nm is proportional to the G6PD activity of the specimen.

**Complete blood count**

The complete blood count was realized by using the blood counter Abacus 4.

**Triglycerides assay**

Triglyceride has been assayed using the enzymatic colorimetric method; according to the technical user manual of the Spinreact Kit (Spain). The triglycerides incubated with lipoprotein lipase (LPL) release the glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphat (G3P) and adenosine-5-diphosphate (ADP), by the glycerol kinase and adenosine phosphate (ATP). The G3P is then converted by the glycerol phosphate dehydrogenase (GPO) in active dihydroxyacetone phosphate (DAP) and hydrogen peroxidase (H₂O₂). The latter reacts with 4-aminophenazone (4-AP) and p-chlorophenol in the presence of peroxidase (POD) to give a red color (Bucolo and David 1973).

**HDL Cholesterol assay**

The dosage of high density lipoproteins HDL has been carried out by the enzymatic method (Spinreact Kit, Spain). The very low density (VLDL) and low density (LDL) lipoproteins were precipitated by phosphotungstate in the presence of magnesium ions. After centrifugation the supernatant contains high density lipoproteins (HDL). The HDL cholesterol fraction was determined using the total cholesterol enzymatic reagent (Naito, 1984; Grove, 1979).

**LDL cholesterol assay**

The dosage of low density lipoproteins (LDL) was assayed according to technical guide of Spinreact Kit, Spain. Direct determination of serum LDL (low-density lipoprotein cholesterol) levels was carried out without the need for any sample pre-treatment or centrifugation of the sample (Friedewald et al., 1972).

**Cholesterol assay**

The assay of high density lipoproteins (HDL) was realized by the enzymatic method according
to the technical data sheet of the Spinreact Kit, Spain (Naito, 1984).

RESULTS AND DISCUSSION

Hematological markers are presented in table 1 showed a significant decrease in Cu group in G6PD, RBC, HGB and HCT levels, contrary Cu has augmented the WBC, PLT and MCV levels compared to the control. No change in the group treated with Cu F, while Cu L group showed a significant increase in G6PD, RBC, HCT and MCV levels, and a significant decrease in WBC level compared to the control.

Table 2 represents the rat’s lipid profile exposed to copper for one month. Our Results showed significant decreases in triglyceride and cholesterol, while HDL and LDL levels kept the same levels compared to the control. Cu F showed an augmentation in triglyceride, cholesterol and decreased the HDL and LDL levels compared to the Cu group. In the group treated with Cu L, triglyceride, cholesterol and HDL levels augmented significantly, while no change was observed in LDL level compared to the Cu group.

In this research, copper sulfate administrated to rat for one month decreased significantly the G6PD, RBC, HGB and HCT levels, while it increased WBC, PLT and MCV. Recently, high copper level decreased the RBC counts and HGB concentration in rats (Akomolafe et al., 2014) as a result of erythrocytes hemolysis induced by the free copper ions. The low activity of G6PD in rats of the copper group might be related to the inhibition of the enzyme by copper ions, an enzyme responsible of the red cells protection from oxidative stress by maintaining the GSH level through NADPH generation (Joshi et al., 2002). Moreover, toxic copper was reported to induce hemolysis, leading to red blood dysfunction (Savaru et al., 2007), and disturbs the erythropoiesis by affecting iron metabolism in the intestinal tracts, where copper and iron are antagonists (Pmila et al., 1991). The observed iron deficiency during high copper level has led to anemia (Eck and Wilson, 1989) and methaemoglobinemia (Oldenquist and Salem, 1999; Ahasan et al., 1994), which confirm that copper is involved in the erythropoiesis process (Samanta et al., 2011). On the other hand, the MCV

Table 1: Mean ± SD of some hematological markers in the different groups after treatments by copper sulphate and C. monogyna for one month.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cu F</th>
<th>Cu L</th>
</tr>
</thead>
<tbody>
<tr>
<td>G6PD (mU/10^9)</td>
<td>121.4±0.9b</td>
<td>83.3±0.6d</td>
<td>124.1±0.8a</td>
</tr>
<tr>
<td>WBC (10^3/mm)</td>
<td>7.49±0.35a</td>
<td>12.04±0.1a</td>
<td>7.95±0.8a</td>
</tr>
<tr>
<td>RBC (10^6/mm³)</td>
<td>10.36±0.3a</td>
<td>8.22±0.2c</td>
<td>9.86±0.4b</td>
</tr>
<tr>
<td>PLT (10³/mm³)</td>
<td>317±1.7a</td>
<td>899±2.4a</td>
<td>316±20.9c</td>
</tr>
<tr>
<td>HGB (g/L)</td>
<td>151.8±5.04a</td>
<td>133.8±3.4c</td>
<td>152±3.6a</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>51.04±0.8b</td>
<td>40.7±0.8c</td>
<td>51.1±0.7b</td>
</tr>
<tr>
<td>MCV (fI)</td>
<td>51.6±0.5c</td>
<td>39.3±0.8c</td>
<td>51.1±0.7b</td>
</tr>
</tbody>
</table>

Means that do not share the same letter are significantly different (p<0.05), according to one-way ANOVA, followed by Tukey test. G6PD: 6-phosphate; WBC: white blood cells; RBC: red blood cells; PLT: platelets, HGB: hemoglobin; HCT: hematocrits; MCV: mean corpuscular volume.

Table 2: Mean ± SD of Biochemical markers in the different groups after treatments by copper sulphate and C. monogyna for one month.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cu F</th>
<th>Cu L</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIG (g/l)</td>
<td>0.88±0.07a</td>
<td>0.13±0.03b</td>
<td>0.63±0.03b</td>
</tr>
<tr>
<td>CHOL (g/l)</td>
<td>0.68±0.007a</td>
<td>0.21±0.01c</td>
<td>0.46±0.01c</td>
</tr>
<tr>
<td>HDL (g/l)</td>
<td>0.31±0.01c</td>
<td>0.31±0.01d</td>
<td>0.56±0.008a</td>
</tr>
<tr>
<td>LDL (g/l)</td>
<td>0.18±0.008b</td>
<td>0.17±0.01ab</td>
<td>0.10±0.005c</td>
</tr>
</tbody>
</table>

Means that do not share the same letter are significantly different (p<0.05), according to one-way ANOVA, followed by Tukey test. TRIG: triglycerides; CHOL: cholesterol, HDL: high density lipoproteins; LDL: low density lipoproteins.
and HCT level have increased significantly when rats exposed to copper, without affecting RBC count and HBG concentration (Akomolafe et al., 2016). Liver injury by the copper toxicosis is may lead to coagulation cascade (Nelson, 2002); this perhaps explains the observed rise in PLT levels in our finding, which was not the case in the study of Ganong, (2009) who reported that high copper charge had decreased the PLT level by inhibiting the thrombopoietin production. Copper may cause inflammatory reactions to some organs such as liver, heart and kidneys, which may explain the increase of WBC as the macrophages that are sensitive to heavy metals toxicity (Witeska and Wakulska, 2007).

The combined treatment of copper and hawthorn fruits extract in this study showed an increase in G6PD activity and HCT levels, without affecting the other parameters. Thus, C. monogyna seems to play an important role in free radicals scavenging induced by copper sulphate, as the study of Bernatoniienė et al. (2008), who indicated that aqueous and ethanolic extracts have the capacity in protecting cells from oxidative stress. Moreover, hawthorn was reported to be rich in polyphenols (Liu et al., 2019), that have protective activity for hematological markers against lead toxicity (Aksu et al., 2012). Also, the active compounds in C. monogyna seem to have the ability to enhance the antioxidant system by rising G6PD activity to protect red blood cells against stress injuries. This enzyme is the main supplier of protons through the coenzyme NADP to generate reduced glutathione.

The remarkable triglycerides and cholesterol concentrations decrease in rats having toxic copper dose after thirty days consecutive exposure were in conformity with the studies of Mondal et al., (2007) and Babaknejad et al., (2015). The maintaining level of LDL and HDL in this investigation was probably linked to the HDL synthesis from LDL via the modulation of HMG-CoA reductase activity by copper (Mondal et al., 2007). Contrary, copper administration to cows (40mg/kg) had led to a cholesterol concentration increase (Engle et al., 2001) and cholesterol and LDL in rats as results of the oxidative stress (Galhardi et al., 2004).

The C. monogyna administration in both L and F groups has reduced the triglycerides levels, cholesterol, and LDL, while it raised the HDL production. In fact hawthorn given to rats at 2% of the diet was demonstrated to have a hypocholesterolemic and vasoprotective activities (Kwok et al., 2010). Researchers found that the alcoholic extract of the C. monogyna berries lowered significantly the cholesterol, triglycerides and the LDL levels (Kausar et al., 2011). Furthermore, C. monogyna could increase the receptors capacity to bind to LDL and therefore prevent the cholesterol augmentation (Kausar et al., 2011) and enhancing the cholesterol elimination to bile (Rajendran et al., 1996). Hawthorn was also been found to decrease the serum levels of cholesterol, LDL-cholesterol, and triglycerides in hypercholesterolemic and atherosclerotic animals (Chang et al., 2002). Also studies showed that hawthorn may lower the body weight as our results indicated, and it used to treat obesity and weight control (Kausar et al., 2012).

In the combined group Cu L and Cu F, the HDL level increased significantly, which means that the hawthorn has a beneficial effect, explained by the presence of catalytic metal ions, that increase the long and short chain cholesterol ester and phospholipids (Abuja and Albertini 2001). While LDL decreased significantly particularly HDL with high copper concentration perhaps by accelerating the LDL oxidation (Raveh et al., 2001).

**CONCLUSION**

The copper induced rat toxicity during thirty days has disturbed most blood parameters and lipid profile, while the co-administration of C. monogyna leaves and fruits extracts has led to a mitigating effect by normalizing many blood biomarkers.

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