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The aqueous extract of fruits and leaves of *Crateagus monogyna* Jacq. in mitigating copper sulphate-induced hepatotoxicity and nephrotoxicity of Wistar rats

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ABSTARCT

This study explores the promising mitigating activity of fruits (F) and leaves (L) aqueous extract of Crateagus monogyna Jacq. (Fam. Rosaceae) against hepatotoxicity and nephrotoxicity induced by copper sulphate (Cu). Adult male Wistar rats were divided into the control (C), two positive controls ssupplemented with F (1.5g/kg bw/day) and L (1.5g/kg bw/day) aqueous extract, Cu group (100 mg/kg bw/day),and two other combined groups having the same dosage (Cu+F, Cu+L). The C. monogyna aqueous extracts and copper sulphate were administrated orally for 30 consecutive days, where liver and kidney glutathione (GSH), malondialdehyde (MDA) and glutathione peroxidase (GPx) were evaluated alongside plasma aspartate aminotransaminase(AST), alanine aminotransaminase(ALT),alkaline phosphatase (ALP), urea and creatinine levels. A significant increase in the activity of AST, ALT and ALP and the creatinine level of the Cu group were observed compared to the control, but Cu+F and Cu+L have significantly decreased AST, ALT,ALP, creatinine and urea levels compared to the Cu group. Cu group has respectively increased hepatic MDA concentration, and decreased GSH level and GP x activity compared to the control. The combined treatments (Cu+F and Cu+L)showed a significant decline in MDA concentration, accompanied with significant raise of GSH and GPx levels compared to the Cu group, as well as both positive controls (F and L) demonstrated a significant augmentations of GSH and GPx levels compared to the control. In kidney, Cu group has respectively increased and decreased MDA concentration and GPx activity, but Cu+F and Cu+L have significantly reduced the MDA concentration and raised both GSH level and GPx activity. To conclude, Cu administration to rats has induced hepatotoxicity and nephrotoxicity, while the combination of this metal with the hawthorn aqueous extract have attenuated such toxicity.

***Keywords*:** Copper,Crateagus monogyna, oxidative stress, rat, toxicity.

INTRODUCTION

Copper is an essential trace element for many biological function; it can play a role as an antioxidant enzyme, and as a cofactor in ceruloplasmin molecule (Sharma *et al*., 2005). Copper is found in animals, plants and microorganisms and even in environmental components as water, atmosphere and soils (Stern *et al*., 2007). Moreover, copper enters in many human activities and it is used in industrial, agricultural and medicinal purposes. This huge usage of copper has increased its dispersion in the environment creating a chronic exposure to living organisms and the whole ecosystems.

In the body, copper enters through the digestive tract, respiration, and it even crosses the skin, where nearly 30-50% is absorbed to the blood stream (Turnlund *et al*., 1997), in which the big portion binds to serum albumin (Anant *et al*., 2018). Copper is located principally in the liver, where its metabolism takes place and it mainly excreted by the bile duct. Copper was postulated to activate the Fenton reaction to form reactive hydroxyl radicals leading to cellular damage (Baureder *et al*., 2012). Thus, excess of copper may provoke the deactivation of ATP7B, which might leads to liver, brain and other organs' injuries such as liver cirrhosis, neurological disorders, kidney malfunctions and red blood destruction, known as Wilson's disease (Allen *et al*., 2006). Long term exposure of rats to copper leads to its accumulation in liver, followed by kidney and brain, in which free tissue copper was positively correlated with oxidative stress and organs' dysfunction (Kumar *et al*., 2016). Liver is known to play a key role in maintaining Cu

homeostasis, and also the xenobiotics' detoxification (Chiang, 2014), since all absorbed nutrients pass into the liver by the portal vein.

Through years, the usages of plants as food, and as medical remedies in treating many diseases such as cancer and diabetes were proven by many researchers (Bhowmik, 2019), where, these plants are cheap and available (Momin *et al*., 2018). Hawthorn was demonstrated to have a beneficial effect on human health (Çoklar *et al*., 2018). It is a spontaneous tree of rosaceous family distributed mainly in Africa, Asia, America and Europe (Muradoğlu *et al*., 2019). Hawthorn is being used as a food or a as medicine to treat cardiovascular disorders, stomach troubles, inflammation (Zhang *et al*., 2002), atherosclerotic diseases (Chang *et al*., 2002), respiratory impairments (Arrieta *et al*., 2010) and as an antioxidant (Osawa, 1994). Crataegus monogyna was reported to contain iron, zinc, manganese, magnesium (Özcan *et al*., 2005), in addition to oxalic acid, malonic acid, palmitic acid, oleic acid, linoleic acid, and other essential oils (Bechkri *et al*., 2017). As a result, the presence of flavonoids, chlorogenic acid and Triterpenes make it a powerful antioxidant useful to many medicinal treatments (Nabavi *et al*., 2015).

The present work explores the possible mitigating activity of Crataegus monogyna fruits and leaves aqueous extracts against the chronic toxicity of copper through the evaluation of hepatic and renal markers of male Wistar rat.

MATERIAL AND METHODS

Plant and copper preparation

The common hawthorn trees *Crataegus monogynaare* grown spontaneously along the northern zone of Algeria, exceeding 3 meters in length, and characterized by green leaves, white flowers and red fruits; the latter reaches maturity in mid-autumn. The plant authentication was made by the staff of the Department of Biology, in which voucher specimen were deposited in the laboratory. Fruits and leaves of C. monogyna were harvested freshly in November from Annaba area, northeast Algeria. Therefore, fruits and leaves were separately weighted daily, crushed in 20 ml of distilled water and were kept overnight (12 hours) at room temperature. Then, the homogenates were filtered in the morning to obtain the aqueous extracts of F and L, where rats receive the equivalent of 1.5g/kg bw/day of the filtered extract obtained. 100 mg of copper sulfate pentahydrate salt ($CuSO4 \cdot 5H2O$) was dissolved daily in distilled water. The combined treatments were made by mixing volumes of dissolved copper sulphate and the filtered aqueous extracts (Cu+F, Cu+L), which immediately administered to animals by gavage; this procedure is repeated every day during 30 days.

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/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 Cu sulphate/Kg bw + 1.5 g fruits/kg bw) and the Cu+L(100 mg Cu sulphate/Kg bw + 1.5 g leaves/ kg bw) group. Rats were sacrificed by decapitation after 30 consecutive days of oral administration of copper solution and fruits and leaves aqueous extracts. Blood was received in heparinized test tubes, was immediately centrifuged at 3000 rpm for 10minutes, and then the plasma was stored at -20 °C together with the liver and the kidney till further analysis. Animals' experiments were authorized by the Ethical Committee of Animal Sciences at the University of Badji Mokhtar-Annaba.

Plasma markers assay

The dosage of asparatateaminotransaminase (AST), alanine aminotransferase(ALT), alkaline phosphatase (ALP),urea and creatinine have been carried out by the colorimetric method according to the technical data sheet of the SpinreactKit,Spain.

AST catalyses the transfer of an aspartate moiety to alpha-ketoglutarate to form glutamate and oxaloacetate, the latter is reduced to malate in the presence of dehydrogenated (MDH) and NADH (Murray, 1984).

In the ALTreaction, an amino group from alanine is transferred to α-ketoglutarate forming glutamate and pyruvate thatis reduced to lactate by lactate dehydrogenase (LDH) and NADH (Murray, 1984).

Fig. 1: Fruits and Leaves of Crateagus monogyna Jacq. collected in 2018.

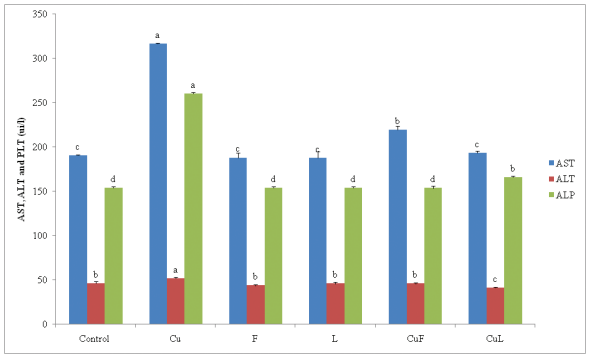

Fig. 2: Plasma variation (mean±SD) of AST, ALT and ALP activity) levels of rats treated by copper, C. monogyna and the combination of copper and hawthorn for 30 days. Means that do not share the same letter are significantly different at p<0.05.

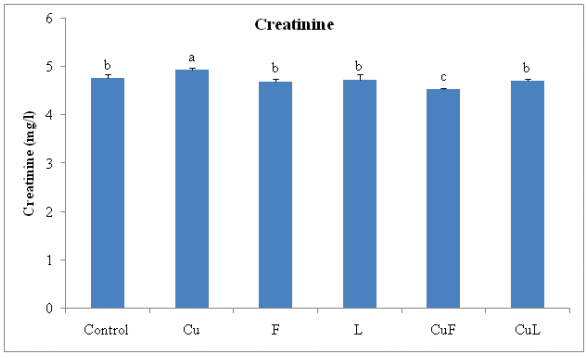

Fig. 4: Plasmavariation (mean±SD) of creatinine concentration of rats treated by copper, *C. monogyna* and the combination of copper and hawthorn for 30 days.Means that do not share the same letter are significantly different at p<0.05.Means that do not share the same letter are significantly different at p<0.05.

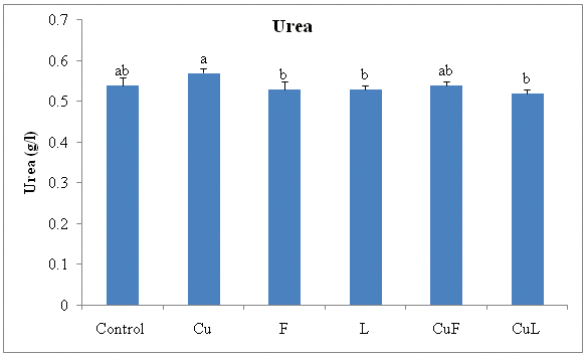

Fig. 3: Plasma variation (mean±SD) of urea concentration of rats treated by copper, *C. monogyna* and the combination of copper and hawthorn for 30 days. Means that do not share the same letter are significantly different at p<0.05.

ALP catalyses the transfer of the phosphate groupfrom p-nitrophenylphosphate (pNPP) to 2-amino-methyl-1 propanol by releasing p-nitrophenol and phosphate (Wenger *et al*., 1984; Rosalki *et al*., 1993).

Urea in the sample is hydrolyzed enzymatically into ammonia (NH4+) and carbon dioxide (CO2). Ammonia ions formed reacts with salicylate and hypochlorite (NaClO), in presence of the catalyst nitroprusside, to form a green indophenol sample (Kaplan, 1984).

Creatinine reacts with alkaline picrate forming a red complex. The intensity of the color formed is proportional to the creatinine concentration in the sample (Murray *et al*., 1984).

Oxidative stress assay

100mg of frozen liver and kidney were thawed and transferred to test tubes for the determination of glutathione reduced (Weckbecker and Cory, 1988), total proteins by using the Coomassie Brilliant Blue G-250 (Bradford, 1976), malondialdehyde (Ohkawa *et al*., 1979), and glutathione peroxidase (Flohe and Günzler, 1984).

Statistics

Statistics was appliedby using ANOVA followed by Tukey test (MINITAB 18 Software). Results are expressed as mean ±standard deviation. The significant test was considered at $p<0.05$.

RESULTS AND DISCUSSION

Copper intake has significantly increased the AST and the ALT activity compared to the control, meanwhile these enzymes were significantly decreased in the CuF and CuL groups compared to the Cu-treated group, while the positive control F and L have maintained the same activity of AST and ALT as that of the control (Figure 2).

The ALP activity of the Cu group showed a significant increase when compared to the control, but it decreased significantly in the CuF and CuL groups compared to the Cu group (Figure 2).

Liver enzymes are usually used to detect the hepatocytes' damage. In this study, the increase of plasma AST, ALT and ALP activities was noticed in animals intoxicated with copper for thirty days, such result is in line with that reported by Emad and Shimaa, (2016) in serum rats, and with that of urinary AST of male rats after the treatment with 140 mg Cu/kg/day of copper (Anonymous, 1993). Other previous studies have confirmed that copper sulphate may lead to liver cancer (Tchounwou *et al*., 2008), which probably originated from the deficiency of zinc and vitamin B6 during copper intoxication (Eck and Wilson, 1989). Moreover, Cu supplementation that induced liver cirrhosis and hepatocytes necrosis (Winge and Mehra, 1990) agrees with the augmentation of enzymes' activity observed in this study. Cell injury following copper accumulation was reported to be caused by ROS generation (Manzl *et al*., 2004; Emad and Shimaa, 2016), where a positive correlation was confirmed by liver free copper concentration and organ dysfunction (Kumar *et al*., 2016) in rats supplemented with 100 and 200mg/Kg bw/day. In contrast, Wistar rats exposed to copper (60mg Cu/kg bw) for four weeks and also young people (0.315 mg Cu/kg/day) for nine months did no demonstrate any variations in serum ALT and AST activities (Galhardi *et al*., 2004; Zietz *et al*., 2003).

Urea level has a slight increase in rats exposed to Cu compared to the control, but it decreased significantly in the CuL group compared to the Cu group (Figure 3).

Results of creatinine concentration of rats exposed to Cu toxicity demonstrated a significant increase compared to the control, but it decreased significantly in the CuL and CuFgroups compared to the Cu group (figure 4).

Plasma urea and creatinine concentration are used to follow the kidney excretion status. Therefore, urea level has not been affected in animals exposed to copper for one month, while creatinine showed a remarkable increase, indicating the possible copper toxic effect. Alongside, urea plasma level was increased in rats' experienced copper intoxication (Akomolafe *et al*., 2016), as well as the observed relationships between Cu exposure and human renal disorders (Sinkovic *et al*., 2008). Furthermore, blood urea nitrogen level was clearly correlated with kidney free copper content in rats exposed to copper for 90 days (Kumar *et al*., 2015). Contrary, 50 µmol/kg bw of inorganic copper for 30 days have not made any observed changes in the concentration of blood urea and creatinine in Wistar rats (Abou-seif *et al*., 2003).

Hepatic MDA level demonstrated a significant increase in Cu-exposed group compared to the control, while the positive controls of C. monogyna F and L extracts have almost the same level as that of the control.

On the other hand, hepatic GSH concentration and GPx activity were decreased significantly by Cu treatment compared to the control, with a remarkable increase in the combined treatment CuF and CuL compared to the Cu group. However, the positive controls of F and L have showed a

significant rise in the levels of GSH and GPx compared to the control.

Renal MDA concentration was increased significantly on the Cu group compared to the control, although it has been decreased significantly in rats of the CuF and CuL groups compared to the Cu group. The positive groups F and L were not significantly different than that of the control.

Renal GSH level and GPx activity have been decreased significantly in the Cu group compared to the control, but when compared to the Cu group, the two markers of CuF and CuL groups were highly significant. Interestingly, the positive group F of hawthorn extracts hashigher GSH and GPx levels than that of the control.

High copper content in cells could create an imbalance between oxidative stress production and the antioxidant defense system (Sies, 2015), as that of Haber-Weis is reaction, which can be activated by metal toxicity to generate reactive oxygen species (Rosario *et al*., 2017), leading to mitochondrial dysfunction (Myers *et al*., 1993). As a result, copper may provoke cell membrane damages through the augmentation of lipid peroxidation that could lead to liver and kidney cells' necrosis, which might be explained by the increase of hepatic and renal MDA concentration in rats administrated with copper sulphate during four consecutive weeks. Such result is in-line with that of Kumar *et al*. (2016) who demonstrated an augmentation of hepatic and renal MDA level in parallel with the tissue free copper after exposure of rats to copper sulphate during three months.

The remarkable decrease of hepatic and renal GSH and GPx levels after one month exposure to copper is likely affected by ROS generated by copper ions, exactly as what was reported previously, where GSH concentration was decreased in rat after copper sulphate injection (Ossola *et al*., 1997), and also the observed decrease in hepatic GSH level and GPx activity after Cu overload in rats (Rosario *et al*., 2017). Thus, copper ions were demonstrated to decrease GSH concentration due the high affinity of sulfhydryl group to this metal (Rosario *et al*., 2017), and also glutathione concentration of liver was inversely correlated with serum aminotransaminases and tissue free copper of rats intoxicated with copper (100 and 200mg/Kg bw) during 90 days (Kumar *et al*., 2016). Certainly, copper ions accumulated in liver and kidney during this experiment are highly attracted to sulfhydryl groups, which leads to lowering the level of glutathione and by consequent induces a decrease in GPx activity. Indeed, copper ions accumulated in liver and kidney of the exposed rats are highly attracted to sulfhydryl groups, which leads to lowering the level of glutathione and by consequent induce a decrease in GPx activity (Rosario et al., 2017).

From the data obtained in this investigation, the previous markers of rats supplemented with the combined treatment of copper and hawthorn were almost within the normal physiological ranges, that to see *C. monogyna* fruits and leaves extracts have the capability to mitigate the disturbing action of copper ions. The likely reason is that C. monogyna is rich in many components as vitamin C and phenols (Muradoğlu *et al*., 2019), probably this is why it is used as an antioxidant to treat certain diseases (Osawa, 1994). This could explain the role played by *C. monogyna* in strengthening the antioxidant system through the augmentation of hepatic and renal GSH and GPx levels. Accordingly, supplementing *Crataegus pinnatifida* leaves extract to rats have demonstrated a significant increase in antioxidant enzymes (Wang *et al*., 2011). Moreover, it was found that the presence of linoleic, oleic and palmitic acids in *C. monogyna* could enhance the antioxidant activity (Bechkri *et al*., 2017), and also may reduce copper intestinal absorption since hawthorn was noted to be rich in vitamins, zinc, and iron, which might antagonist copper ions intake (Ozcan *et al*., 2005). Therefore, the mitigating benefit of hawthorn is likely established by scavenging the superoxide anions, hydroxyl radicals, hydrogen peroxides and reducing lipid peroxidation (Rice-Evans, 2004).

CONCLUSION

The aqueous extract of fruits and leaves at the dose of 1.5 g/kg bw administered orally to Wistar rats have proved to have mitigating activity towards

liver and kidney markers' disturbances induced by copper for thirty days.

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