Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Effect of Spirulina-Platensis against Nano-copper Toxicity in Rabbit

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Received in 11/11/2021
Received in revised from 29/11/2021
Accepted in 22/12/2021

Keywords:

Spirulina platensis
Nano-copper particles
oxidative stress
rabbits

ABSTRACT

The present study aimed to monitor the antioxidant and protective effects of Spirulina platensis (SP) against Nano- copper particles (NCPS) which induced liver injuries in rabbits. The experimental animals were randomly divided into three groups, the first group, (control), the second group, (received NCPS, 37.5 mg/kg), and the third group, received NCPS, 37.5 mg/kg bw plus SP, 1.5 g/kg diet for 2 months. After ending the period of the experiment, rabbits were decapitated and, blood, liver, as well as muscle tissue samples were collected for examination of liver function, antioxidant activity, lipid peroxidation, and copper levels. Exposure of rabbits to these Nanoparticles S\C for 8 weeks resulted in significant increase levels of glutathione (GSH) in the third group. While hepatic superoxide dismutase (SOD) and malondialdehyde (MAD) activity showed a significant decrease in their levels of the same group. In addition, alanine aminotransferase (ALT) in serum showed a significant decrease. These data revealed that administration of SP protects against Nano copper-induced hepatotoxicity through amelioration of hepatic damage and oxidative stress in rabbits.

INTRODUCTION

Heavy metals in air, soil, and water represent considerable problems for humanity. These heavy metals are widespread in the environment, as they contaminate the food, air, water and accumulate in the tissues in high proportions **(Al-Attar, 2011).**

Many recent studies have been conducted on Nano-metric copper particles because of their great importance in nanotechnology **(Hejazi et al. 2018 and Wang and Zhang, 2019).** Since the information about Nano metric copper particles and their toxicity is limited and incomplete until now. It is necessary to know the toxicity of these nanoparticles. They may be lead to pollution in the environment and many health risks as they are used in medicine, therefore identifying their toxicity before use in many modern applications, is of great importance **(Ahamed et al. 2015).**

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DOI: 10.21608/EJAH.2022.225148

Copper itself is less toxic than NCPS due to the mechanisms of NCPS toxicity, which has not been completely identified. Two major factors revealed their toxicity. Firstly, they act as potent oxidative and catalytic agents, the Nano copper catalytic and oxidative actions are not blocked due to its storage and transportation is not proteins-like as in the case of the copper element. Secondly, metabolic processes (detoxification reactions in different phases) and, elimination of copper particles are difficult because of their chemical and physical properties, as compared to copper metal **(Pohanka, 2019).**

Copper is an important element in technology such as electronic technology because of its electrical and thermal conductivity **(Khalaj et al. 2018 and Deka et al. 2019).** Many uses of copper make it easy to enter and affect the environment and the health of all populations. Although copper has little toxicity, deposition of copper in the body causes some diseases **(Dusek et al. 2015 and Deforest et al. 2018).**

Copper is involved in many metabolic processes in the living organism. Therefore, it is an important biological element. Intake of the copper element in low dose causes a problem, while, overdoses cause toxicity **(Pohanka, 2019).** The major affected organ of copper toxicity is the liver, where the toxicity of copper leads to many diseases like "Indian childhood, “idiopathic-Copper toxicities” and "Tyrolean infantile cirrhosis" **(Muller et al. 1998).**

SP is a unicellular cyanobacterium with high nutritional value and wide range of medicinal applications. It contains a very potent naturally occurring antioxidant that has free radical scavenging and antioxidant activities. SP and its active constituent, “c-phycocyanin” exhibit, anti-inflammatory, neuroprotective, hepatoprotective, immune-modulatory, and anticancer activities **(Reddy et al. 2000 and Basha et al. 2008).** It has been found that SP has antioxidant effects against metal toxicity in many animal species **(Soliman et al. 2021).** As it contains organic components with antioxidant properties that protect cells and tissues from the harmful effect of reactive oxygen, species and other free radicals **(Eidelman et al. 2002).** Furthermore, SP has a reported ameliorating effect against organ toxicities induced by heavy metals **(Simse et al. 2009 and El-Desoky et al. 2013).** Increased interest in SP as it is non-toxic, bioavailable, and gives multi-organ protection against many drugs and chemicals induced toxicity **(Khan et al. 2006 and Lu et al. 2010)**.

The high value of spirulina constituents leads to their uses as cosmeceuticals, nutraceuticals, pharmaceuticals, and functional foods **(Borowitzka, 2013).** In addition, several toxicological studies have been conducted on SP, and it has been shown that SP does not have any toxic effect when taken **(Yang et al. 2011).** Therefore, SP has been used to prevent and treat hepatic diseases especially those induced by oxidative damage. Many previous studies showed the hepato- protective effects of Spirulina platensis and its active constituents against drugs, chemicals, and xenobiotic. In addition, the antioxidant effects of SP have been reported against cadmium, which was revealed by lowering of MDA as well as increasing of GSH and SOD activity in liver tissue **(Vadiraja et al. 1998 Karadeniz et al. 2009 and Ismail et al. 2009).** Consequently, the aim of the present study was to investigate the hepatoprotective and antioxidant effect of Spirulina platensis against Nano copper particles induced oxidative stress and hepatotoxicity in rabbits.

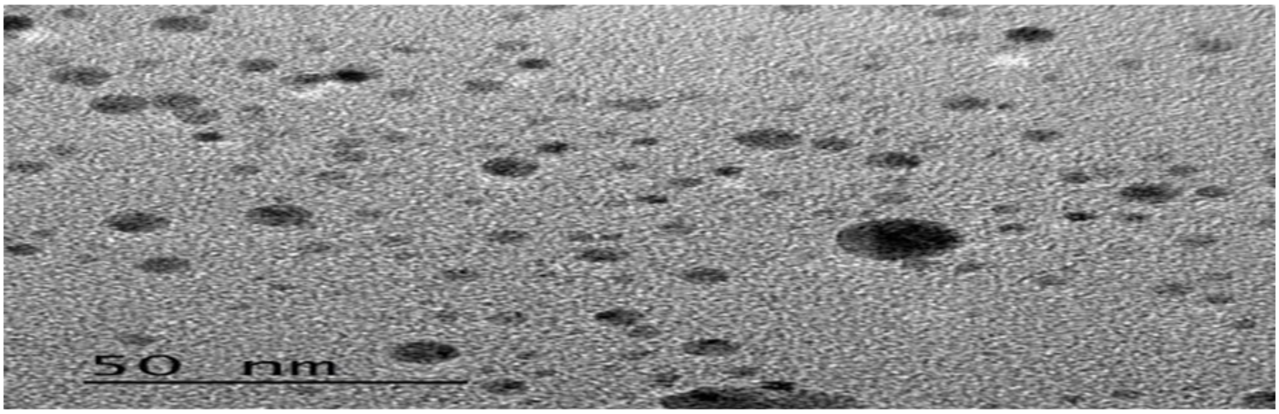
MATERIALS AND METHODS:

Animals

Fifteen rabbits, 35 days old with 633 g average body weight, were used for the present study. All rabbits were acclimatized in metal batteries with equal light and dark cycles and constant environmental conditions (Temperature 25±2 ºc and moderate humidity). The animals were provided with a basal rabbit diet and water ad libitum throughout the experiment.

Chemicals

CNPS (50 nm) at 90% purity were prepared in Nanotechnology unit, Faculty of pharmacy Cairo university according to the method of (**Zhu et al. 2005).**



TEM (transmission electron microscopy) analysis of NCPS using a conventional instrument shows a typical low-resolution image of copper Nano-particles showing a core /shell structure. In addition, the obtained size distribution from the low-resolution image of the sample is 50 nm. LD50 for CNP-induced toxicological effects and injuries for liver tissue is 375 mg/kg S/C in rabbits, the selected dose of CNPS (37.5 mg/kg bw. 1/10 LD_{50}) used in the present study (**Scientific Committee on Consumer Safety "SCCS", 2021).** Spirulina platensis (SP) powder has purchased from Algae Biotechnology Unit at the National Research Center. The selected dose of SP is 1.5 g/kg in diet according to **Aladaileh et al. (2020).**

Experimental design

After 1 week, rabbits were divided into three experimental groups (5 each). The first group served as a control and received a normal diet without SP. The second group received 37.5 mg/kg of Nano copper particles S/C for 5 days /weekly for 8 weeks without SP in their diets. Animals of the third group were exposed to the same dose of CNPS as described before in the second group, with SP as 1.5 g/kg in their diet.

Biochemical analysis

After the end of the experiment blood samples were collected. Half of the blood samples were collected for copper evaluation according to the method conducted by **Zheng et al. (2009).** The other half centrifuged at 10,000 rpm/min. for ten min, at four ºc for serum collection. Serum was then stored at -20º c for further serum alanine aminotransferase activity according to the method conducted by **Reitman and Frankel (1957).** The tissue of the liver, was homogenized in "phosphate buffer saline" (pH 7.4), and the obtained tissue homogenate was centrifuged at 10.000 rpm/min for fifteen min, and after that, it was used for the evaluation of superoxide dismutase enzymes (SOD) activity according to the method conducted by **Ellman(1959),** malondialdehyde, (MDA) concentrations according to the method described by **Ohkawa et al. (1979).** In addition reduced glutathione (GSH) levels according to the method revealed by **Aebi (1984)**.

Estimation of copper content in liver and muscle tissue samples

Liver and muscle tissues have been digested with a nitric acid solution-using microwave then; the concentration of copper in digested fluids has been analyzed using an atomic absorption spectrometer according to the method described by **Zheng et al. (2009).**

Statistical analysis

The obtained results were expressed as mean ± SD. All data were analyzed using (ANOVA) one-way analysis of variance according to SPSS version 16.0 statistical software **(SPSS, Inc, Chicago, IL. 2007).** The difference was significant when P ≤0.05.

RESULTS

1-Serum biochemical analysis

Data analysis in figure- (1). Revealed the mean values of serum ALT activity (U/L) which were 32.78 ± 1.86, 76.2 ± 1.92, and 55.46 ± 1.43 in the three treated groups respectively. These data showed that the Nano-copper treated group has a significant (P≤0.05) increase in serum ALT levels in comparison with the control group. While the third group which, received both Nano-copper and Spirulina platensis (as an antioxidant) showed a significant decrease in serum ALT levels compared to the Nano-copper treated group and with a slight non-significant increase compared to the control group. Therefore, data confirmed that the SP restored the normal levels of serum ALT in Nano-copper intoxicated rabbits.

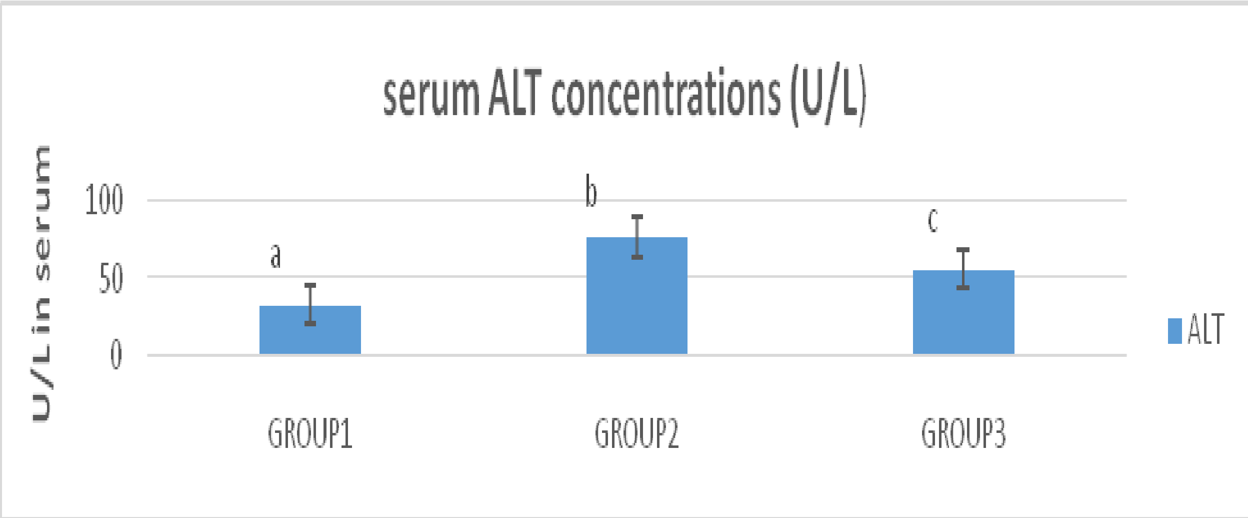


Figure (1) the mean values of serum ALT (U/L). Data expressed as mean ±SD in the control and the two treatment groups of rabbits in the experiment (n=5). Group-1(control), group- 2 (Nano-copper treated) and group-3 (Nano-copper plus SP treated group). [Abc] Values with different superscripts within the same figure are significantly different (P≤ 0.05)

2-liver lipid peroxidation and antioxidant biomarkers:

Data analysis in figure (2). Revealed that the mean values of SOD [a] superoxide dismutase (U/mg), MDA [b] malondialdehyde (µm/mg), and GSH [c] glutathione peroxidase ((µm/mg) tissue) were, 267.88±2.38, 298.6±4.88 and 270.11±4.18 in SOD, 6.43±1.54, 11.166±1.94 and 7.078±1.85 in MDA, and 5.577±1.59, 3.962±1.244, and 5.133±1.481 in GSH in the control and the two treatment groups respectively. These data showed that Nano-copper treated group has an extremely significant (P≤0.05) increase in SOD activity and MDA levels compared to the control group. While a group that received, Nano-copper and SP showed significant reductions in liver SOD activity and MDA concentrations in comparison to the second group (Nano-copper treated group) and with a slight non-significant increase in comparison with the control group. In addition, data analysis revealed that Nano-copper treated group, (second group) showed an extremely significant reduction in liver GSH concentration in comparison to the control group. While the group that received, both Nano-copper plus SP showed a significant (P≤0.05) increase in GSH concentration in comparison to the NC-treated group. Moreover, with a slight non-significant reduction in comparison with a control group.

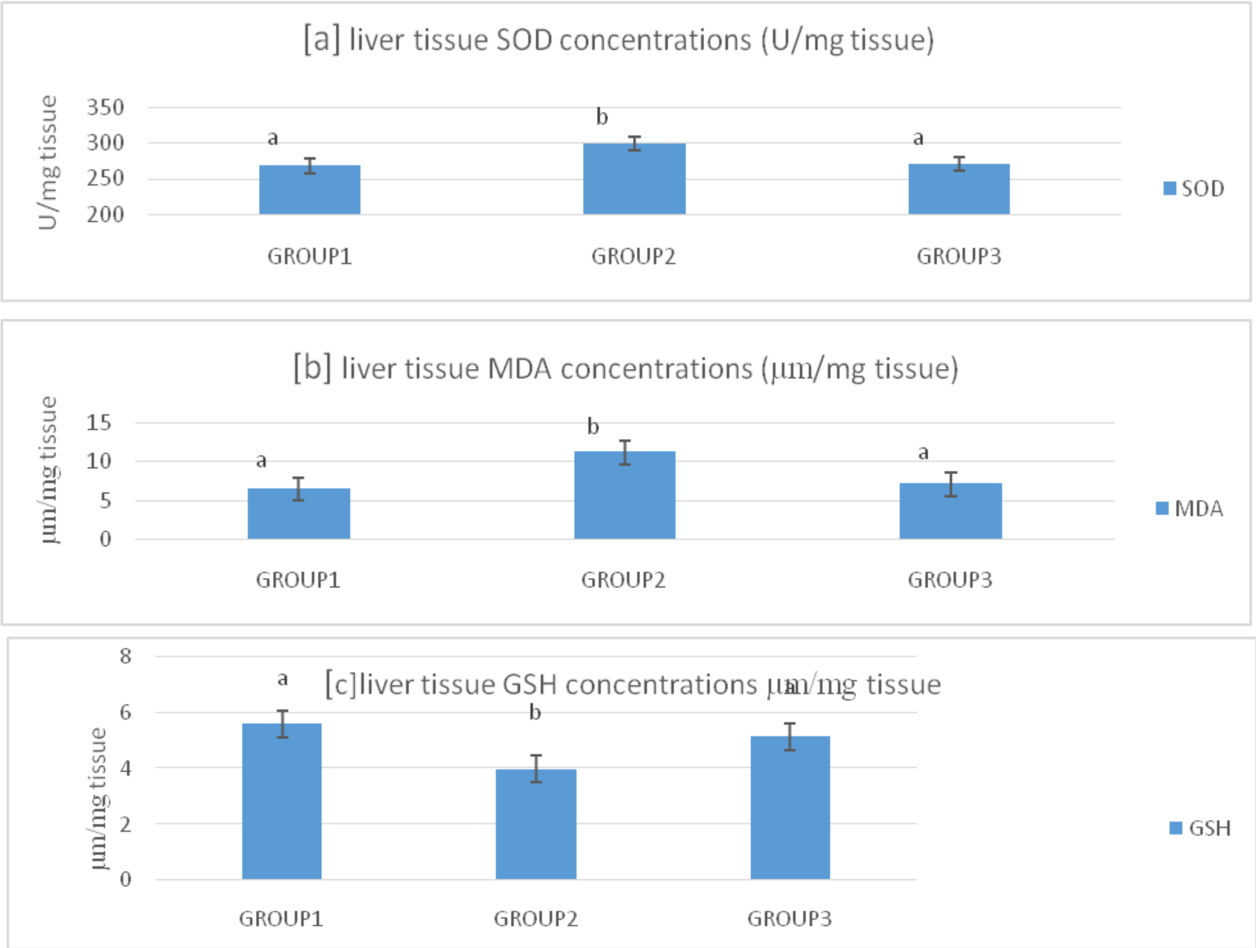


Figure-2.the mean liver values of SOD (U/mg) [a], MDA (µm/mg) [b] and GSH (µm/mg) [c] Data expressed as mean ±SD in the control and the two treatment groups of rabbits in the experiment (n=5). Group- 1(control), group- 2 (Nano-copper treated) and group-3 (Nano-copper plus SP treated group). [ab]Values with different superscripts within the same figure are significantly different (P≤ 0.05).

3-Copper values in different samples (liver, blood and muscle).

Data analysis in figure (3) revealed the mean values of copper in the liver, blood, and muscle (ppm) were 2.83±1.053, 3.317±1.55, and 2.832±1.12 in the liver, 0.573±0.150, 0.874±0.127 and 0.734±0.102 in blood and 2.289±0.69, 2.665±1.077, and 1.987±0.70 in muscle. Moreover, these data analyses in figure- 3 revealed that Nano-copper treated group showed a significant (P≤0.05) increase in copper concentrations compared to the control group in the liver, blood, and muscle. While the third group which received Nano-copper plus Spirulina showed an insignificantly (P≤0.05) decrease in copper concentration in comparison to the second group (NC treated group) in the liver, blood, and muscle and with a slight non-significant increase in comparison with the control group in liver and blood. While in the case of muscle, there was an insignificant decrease in copper concentration in the third group compared to the control group.

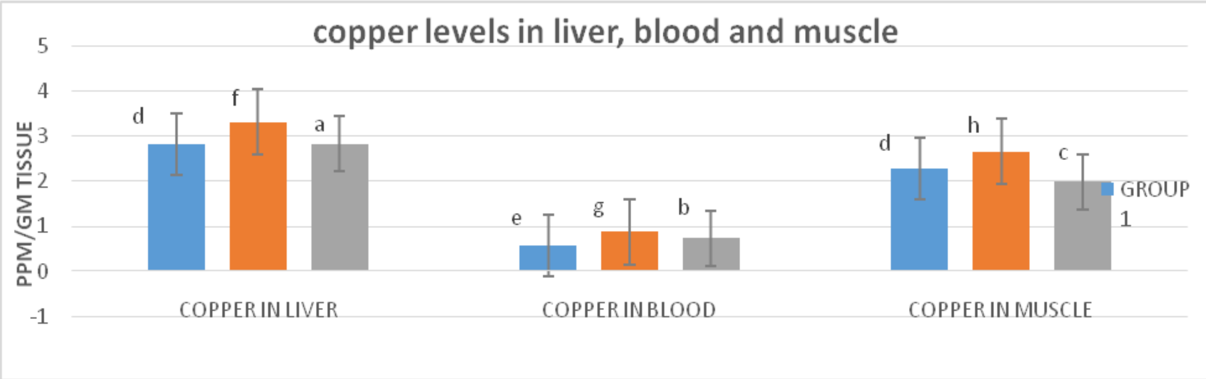


Figure-3. Levels of copper (ppm) in different samples (liver, blood and muscle). Data expressed as (mean ±SD) in the control and the two treatment groups of rabbits in the experiment (n=5). Group- 1(control), group- 2 (Nano-copper treated) and group-3 (Nano-copper plus SP treated group). [abcdefgh]Values with different superscripts within the same figure are significantly different (P≤ 0.05).

DISSCUSSION

Many studies on the biological effects of Nanoparticles show signs of toxicity to the living organisms **(Meng et al. 2007).**Therefore, in this study, the toxicological effects of Nano copper particles in rabbits have been investigated as well as the ameliorative role of spirulina platensis against Nano copper particles in regarding to the biochemical and different antioxidants in intoxicated rabbits. The present study showed that rabbits received NCPS (S\C) in a dose of (37.5 mg\kg. bw.), induced a marked and significant increase in serum activity of ALT (P≤0.05) in the second group of experiment in comparison with the control values figure 1. In addition, this result agrees with that recovered by **Galhardi et al. (2004); Khalaf et al. (2017) and Ebaid et al. (2017)**, who noticed marked liver damage which is correlated with the elevations of serum ALT activity. Therefore, the elevation of serum ALT activity is considered the index of hepatotoxicity. Because of hepatic damage, alteration in the permeability of cell membranes causes liberation of ALT enzyme into the circulation **(Reuben, 2004).**

Co-administration of spirulina platensis causes a significant decrease in the activity of ALT in the "third group" and reduces the toxic effect of Nano copper particles but did not reach the value of the control group. Similar findings reported that SP has a protective effect against metal toxicity in various animal species **(Soliman et al. 2021).** Andhas potent chelating action for heavy metals, in addition, to its antioxidant and free radical scavenging properties **(Rangsayator et al. 2002).** These antioxidant and protective effects of SP are owed to their content of antioxidant active ingredients such as C-phycocyanins, β carotene, vitamins, minerals, proteins, lipids, and carbohydrates **(Upasani and Balaraman, 2003).**

Nigma et al. (1999) illustrated that the reported increasing in production of reactive oxygen species ROS due to copper stress could be attributed to the depletion of antioxidants or the direct action of copper on peroxidation reaction. This overproduction of such ROS induces oxidative stress unless it has scavenged with intracellular antioxidants.

In the present study, oxidative stress has been assessed by measuring MDA level and the activity of the antioxidant enzyme SOD and non-enzymatic antioxidant GSH in rabbit liver tissue figure-2. The concentration of MDA in liver tissue, "a reflection of the endogenous levels of lipid peroxidation" was higher in Nano copper treated groups than the control group. As a result of the production of reactive oxygen species ROS. Therefore, when hepatocytes had been reacted with copper ions, lipid peroxidation is conducted, and that has been manifested by increased production of hepatic MDA and TBARS (Thiobarbituric acid-reacting substances) **(Waggoner et al. 1999).**

Our results are closely correlated with **Kim**

et al. (2000) and Rajesh and Kala (2015), who showed that the level of MDA "lipid peroxidation product" was shown to increase in the liver of the experimented animals with the increase of copper level**.** In our study, the Nano copper treatment was associated with alterations in the oxidant defense system. This has been shown by the significant increase in MDA levels and increases in the activity of SOD. In contrast, the Nano copper administration resulted in a significant decrease in GSH levels in the liver of intoxicated animals in comparison with the control group. These findings were correlated with those mentioned by **Ebaid et al. (2017),** who showed that the Nano copper administration resulted in a significant increase in MDA levels, also these findings explain the ability of Nano copper to stimulate partial hemolysis because of free radical production and lipid peroxidation.

Superoxide dismutase is considered one of the most important enzymes that function as a cellular antioxidant. Therefore, SOD is said to act as the first line of defense against the superoxide radical generated as a by-product of oxidative phosphorylation **(Rajesh and Kala, 2015).** Our results agreed with **Ozcelik et al. (2003),** who revealed that SOD is one of the important enzymes containing copper and copper supplemented animals exhibited increased activity of SOD in the liver. Also, **Irato and Albergon (2005),** demonstrated that the oral administration of copper is correlated with higher SOD activity.

Pourahmad and Brien (2000), confirmed that the protective role of GSH might result from its ability to act as an antioxidant when hepatocytes are reacted with copper. GSH depilation occurs due to excessive GSH consumption during oxidative stress **(Hwang et al. 2002 and Hung et al. 2003). Mohanalakshmi et al. (2015)**, demonstrated that GSH plays an important role in balancing oxidative stress. It protects cell membranes against oxidative damage via inhibition of lipid peroxidation.

In the present study, Co-administration of SP with Nano copper particles induced a marked protective effect against the toxic action of Nano copper, and this protective effect is represented by decreasing the elevation of serum ALT activity to be near to that of the control group. In addition, this protective effect of SP is represented by decreasing in the MDA level and SOD activities and an increase in the concentration of GSH. Moreover, all these parameters were nearly similar to the values of the control group. The obtained result agreed with the findings reported by **Abdel-Daim et al. (2013),** who showed that the administration of SP (500 and 1000 mg\kg) reduces the serum liver enzyme activity. In addition, it reduced the lipid peroxidation of liver tissues. Our findings revealed that there were elevations of liver antioxidant enzymes and glutathione levels due to Spirulina platensis Co-administration.

In our results, the Co-administration of Spirulina platensis with Nano copper particles induced a marked protective effect against the toxic action of Nano copper particles. This effect was revealed by a significant decrease in the copper levels in the liver, blood, and muscle in the third group (2.832 ±1.12, 0.734±0.102 and 1.9874±0.701 ppm) respectively in comparison with those in Nano copper treated group (the second group) (3.317±1.55, 0.874±0.127, and 2.67±1.077 ppm) in the liver, blood, and muscle respectively, figure-3.

Although this study highlighted that Nano copper particles induced hepatotoxicity could be minimized, and prevented to some extent with the prior administration of spirulina platensis the study has some limitations. These limitations come from the claim that histopathological and additional biomarker assessment is needed to evaluate the effectiveness of the overall approach. Additionally, the small sample size we used could have some effect on our results. However, our presented study here provides a first look at how spirulina platensis can minimize and protect against hepatotoxicity resulting from Nano copper particles.

CONCLUSION

In conclusion, the present study confirmed that Spirulina platensis minimized the hepatotoxic effects of Nano-copper particles through its antioxidant activities and that was clear in terms of serum and tissue biochemical changes, antioxidant enzymes activities, and oxidative stress. In addition, the effects of copper-Nano particles on different organisms must be further investigated because of the high toxicity and the seriousness of the pathological diseases that can be occurred by copper Nano-particles.

REFERENCES

Abdel-Daim M, Abuzead SM, Halawa S. 2013. Protective role of Spirulina platensis againstacute deltamethrin-induced toxicity in rats. PLoS One. 8(9):e72991.

Aebi H, Catalase in vitro. Methods Enzymol. 1984. (105): 121–126.

Ahamed M, Akhtar MJ, Alhadlaq HA, Alrokayan, SA. 2015. Assessment of the lung toxicity of copper oxide nanoparticles: Status. Nanomedicine. 10 (15): 2365–2377.

Aladaileh SH, Khafaga AF, Abd El-Hack ME, and Al-Gabri NA. 2020. Spirulina platensis ameliorates the sub chronic toxicities of lead in rabbits via anti oxidative, anti-inflammatory and immune stimulatory properties. Science of total Environment. 20 (701): 134879. doi: 10.1016/j. scitotenv.

AL-Attar AM. 2011. Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi Journal of Biological Sciences. (18):63-72.

Basha OM, Hafez RA, El-Ayouty YM, Mahrous KF, Bareedy MH. 2008. C-Phycocyanin inhibits cell proliferation and may induce apoptosis in human HepG2 cells. Egypt J Immunol (15): 161–167.

Borowitzka MA. 2013. High-value products from microalgae - their development and commercialization. J Appl Phycol 25(3):743 –756.

Deka P, Borah BJ, Saikia H, Bharali P. 2019. Cu-based nanoparticles as emerging environmental catalysts. Chem Rec. (19): 462 – 473.

DeForest DK, Gensemer RW, Gorsuch JW. 2018. Effects of copper on olfactory, behavioral, and other sublethal responses of saltwater organisms: Are estimated chronic limits using the biotic ligand model protective? Environ Toxicol Chem. 37 (6): 1515–1522.

Dusek P, Roos PM, Litwin T, Schneider SA, Flaten TP, Aaseth J. 2015. The neurotoxicity of iron, copper and manganese in Parkinson’s and Wilson’s diseases. J Trace Elem Med Biol.(31): 193–20.

Ebaid R, Elhussain E, El-Shourbagy S, Ali S, and Abomohra AF.2017. Protective effect of Arthrospira platensis against liver injury induced by copper nanoparticles. Orient Pharm Exp Med. (17):203-210.

Eidelman RA, Lamas GS, Hennerkens H. 2002. The new national education program guidelines, Arch. Intern. Med. (162):2033-2036.

El-Desoky GE, Bashandy SA, Alhazza IM, Al-Othman ZA, Aboul-Soud MA. 2013. Improvement of Mercuric Chloride-Induced Testis Injuries and Sperm Quality Deteriorations by Spirulina platensis in Rats. PLoS One 8(3): e59177. doi: 10.1371/ journal.pone.0059177.

Ellman GL. 1959. Tissue sulfhydryl groups. Arch Biochem Biophys (82): 214–226

Galhardi CM, Diniz YS, Faine LA, Rodrigues HG, Burneiko CM, Ribas BO, Novelli EL. 2004. Toxicity of copper intake, lipid profile, oxidative stress and susceptibility to renal dysfunction. Food Chem Toxicol.(42): 2053-2060.

Hejazy M, Koohi MK, Pour ABM, Najafi D. 2018. Toxicity of manufactured copper nanoparticles – a review. Nanomed Res J. 3 (1): 1–9.

Hung YC, Sava VM, Blagodarsky VA. 2003. Protection of tea melanin on hydrazine induced liver injury. Life Sci. (72):1061-1071.

Hwang JM, Wang CJ, Chou FP. 2002. Inhibitory effect of berberine on tert-butyl hydroperoxide induced oxidative damage in rat liver. Arch Toxicol. (76):664-670.

Ismail MF, Ali DA, Fernando A, Abdraboh ME, Gaur RL.2009. Chemoprevention of rat liver toxicity and carcinogenesis by Spirulina. Int J Biol Sci (5): 377–387.

Irato P, Albergoni V. 2005. Interaction between copper and zinc in metal accumulation in rats with particular reference to the synthesis of induced-metallothionein.

Chemico-Biolol Interact. (155):155-164.

Karadeniz A, Cemek M, Simsek N. 2009. The effects of Panax ginseng and Spirulina platensis on hepatotoxicity induced by cadmium in rats. Ecotoxicol Environ Saf. (72): 231–235.

Khalaf AA, Zaki AR, Galal MK, Ogaly HA, Ibrahim MA, Hassan A. 2017. The potential protective effect of alpha-liopic acid against nano copper particle- induced hepatotoxicity in male rats. Human and Experimental Toxicology.36 (9): 881-891.

Khalaj M, Kamali M, Khodaparast Z, Jahanshahi A. 2018. Copper-based nanomaterials for environmental decontamination – an overview on technical and toxicological aspects. Ecotoxicol Environ Saf. (148): 813–824.

Khan M, Shobha JC, Mohan IK, Rao Naidu MU, Prayag A. 2006. Spirulina attenuates cyclosporine-induced nephrotoxicity in rats. J Appl Toxicol. (26): 444–451.

Kim HS, Kwack SL, Lee BM. 2000. Lipid peroxidation and antioxidant enzymes in the blood of rats treated with benzo[a]pyrene. Chem Biol Interact. 127(2):139–150.

Lu J, Ren DF, Wang JZ, Sanada H, Egashira Y. 2010. Protection by dietary Spirulina platensis against D-galactosamine–and acetaminophen-induced liver injuries. Br J Nutr. (103): 1573–1576.

Meng H, Chen Z, Xing GM, Yuan H, Chen C Y, Zhao F, Zhang CC, Zhao YL. 2007. Ultra high reactivity provokes nanotoxicity: explanation of oral toxicity of nano copper particles. Toxicol. Lett. (175):102-110.

Mohanalakshmi S, Ashok Kumar C, Jayaraman R, Pooja M. 2015. Antidiabetic, lipid lowering and antioxidant potential of Girardinia heterophylla in high fat diet and streptozotocin induced diabetic rats. Orient Pharm Exp Med. (15):287–295.

Muller T, Muller Feichtinger H. 1998. Idiopathic copper toxicosis. Am. J. Clin Nutr. 67(5):1082-1086.

Nigma D, Shukla G, Agarwal A, .1999. Glutathione depletion and oxidative damage in mitochondria following exposure to cadmium in rat liver and kidney. Toxicol Lett. (106):151–157.

Ohkawa HN, Ohishi K, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reac tion. Anal Biochem. (95): 351–358.

Ozcelik D, Ozaras R, Gurel Z, Uzun H, Aydin S. 2003. Copper Mediated Oxidative Stress in Rat Liver. Biolog Trace E lem Res. (96):209-215.

Pohanka M. 2019. Copper and copper nanoparticles toxicity and their impact on basic functions in the body Bratish Med J. 120 (6): 397 – 409.

Pourahmad J,Brien PJO. 2000. A comparison of hepatocyte cytotoxic mechanisms for Cu^{2+} and Cd $^{2+}$. Toxicology. 143 (3): 263-273.

Rajesh V, Kala M. 2015. Antiproliferative and Chemopreventive effect of Annona muricata Linn. On Ehrlich ascites carcinoma and Benzo, [a]pyrene induced lung carcinoma. Orient Pharm Exp Med.(15):239–256.

Rangsayator N, Upatham ES, Kruatrachure M, Pokethitiyook P, Lanza G. 2002. Phytoremediation potential of Spirulina (Arthrospira) platensis: biosorption and toxicity studies of cadmium. Environ Pollut. (119):45–53.

Reddy CM, Bhat VB, Kiranmai G, Reddy MN, Reddanna P. 2000. Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from Spirulina platensis. Biochem Biophys Res Commun. (277): 599–603.

Reitman A, Frankel S. 1957.Amer J.Clin. Path. 28 (1):56-63. doi: 10.1093/ajcp/28.1.56.

Reuben A, Hy's Law. Landmarks in Hepatology. 2004. 39(2):574-578. doi: 10.1002/hep.20081.

Scientific Committee on Consumer Safety SCCS. 2021. Opinion on copper (nano) and colloidal copper (nano), SCCS/1621/20 Final Opinion. Toxicological Evaluation, Acute toxicity, page 28.

SPSS. 2007. Statistical software package for the social sciences SPSS Inc. United states of America. Inc., Chicago, IL. Cited by http://www.spss.com.

Simse kN, Karadeniz A, Kalkan Y, Keles ON, Unal B. 2009. Spirulina platensis feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. J Hazard Mater. (164): 1304–1309..

Soliman HAM, Mohamed H, Alaa El-Din H S. 2021. Investigating the effects of copper sulfate and copper oxide nanoparticles in Nile tilapia (*Oreochromis niloticus*) using multiple biomarkers: the prophylactic role of Spirulina. Environmental Science and Pollution Research (28):30046–30057.

Upasani CD, Balaraman R. 2003. Protective effect of Spirulina on lead induced deleterious changes in the lipid peroxidation and endogenous antioxidants in rats. Phytother Res.(17): 330–334.

Vadiraja BB, Gaikwad NW, Madyastha KM. 1998. Hepato-protective effect of C-phycocyanin: protection of carbon tetrachloride and R-(+)-pulegone- mediated hepatotoxicity in rats. Biochem Biophys Res Commun. (249):428-431.

Waggoner D, Bratnikas T, and Gitlin J. 1999. The role of copper in neurodegenerative disease. Neurobiol. Dis. (6):221-230.

Wang S, Lv J, Zhang S. 2019. Discovery of crr1-targeted copper deficiency response in chlamydo monasreinhardtii exposed to silver nanoparticles. Nanotoxicology. 13 (4):447-454.

Yang Y, Park Y, Cassada D, Snow D, Rogers D, Lee J. 2011. In vitro and in vivo safety assessment of edible blue-green algae, Nostoc commune var. sphaeroides Kützing and Spirulina plantensis. Food Chem. Toxicol.(49):1560–1564.

Zheng W, Jiang YM, Zhang YS. 2009. Chelation therapy of manganese intoxication by para-aminosalicylic acid (PAS) in Sprague-Dawley rats. NeuroToxicology. (30): 240–248.

Zhu H, Zhang C, SYin Y. 2005.Novel synthesis of copper nanoparticles: influence of the synthesis conditions on the particle size Nanotechnology. (16):3079-3083.