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Green synthesis and characterization of copper nanoparticles and their effects on liver function and hematological parameters in mice

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Abstract

Introduction: The present investigation was conducted to green synthesize copper nanoparticles (CuNPs) from aqueous extract of *Capparis spinosa* L. fruit and evaluate their effects on liver function and hematological parameters of mice.

Methods: The green synthesis of CuNPs by means of *C. spinosa* extract was performed according to the method described elsewhere. UV-vis spectroscopy analyses, Fourier transform of infrared (FTIR), scanning electron microscopy (SEM), and energy dispersive X-ray (EDX) were used to identify the synthesized nanoparticles. The mice were orally administrated with CuNPs at the doses of 1000, 2000, and 5000 µg/kg for two weeks. Afterward, the effects of CuNPs on liver function of the treated mice were evaluated by measuring the serum levels of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin as well as hematological parameters including hemoglobin (Hb), hematocrit (Hct), white blood cell counts (WBC), red blood cell counts (RBC), and platelet (PLT) counts.

Results: A maximum peak at the wavelength of 414 nm approved the biosynthesis of the copper nanoparticles. FTIR spectrum analysis revealed that the factor groups shaped a coating extract on the surface of the nanoparticles. SEM images demonstrated the particle size between 17 and 41 nm. It was found that although some liver enzymes and hematological parameters increased with increasing dosage of extract, there is no significant difference ($p > 0.05$) between oral administrations of CuNPs at the doses of 1000, 2000, and 5000 g/kg and control group.

Conclusion: The findings revealed that copper nanoparticles biosynthesized from aqueous extract of *C. spinosa* fruit have no toxic effect on the liver of the studied mice; as well as no significant toxicity was observed on hematological parameters in mice. However, more studies need to be done for evaluation of hepatoprotective effect of CuNPs.

Keywords: Nanoparticles; Copper; Liver; Hematology; *Capparis spinosa*; BALB/c mice

1. Introduction

Nanotechnology is one of the most useful technologies that can is applied in many areas including food and nutrition, biomedical science, gene transmission, energy science, electronics, and space industry. In particular, this technology is implemented in the treatment of cancer, allergies, inflammation, diabetes, and other diseases [1]. There are various physical and chemical methods for the production of nanoparticles, which are still being investigated for the purpose of obtaining particles with a certain size and the lower toxicity [2]. Green synthesis is considered as a new approach to prevent the production of undesired or unsafe by-products via the making of reliable, maintainable and eco-favorable synthesis techniques. Between the current green procedures of synthesis of nanoparticles, use of plant extracts is a rather proper and easy method to harvest nanoparticles at large scale relative to bacteria and/or fungi mediated synthesis [2, 3]. Recent studies have also shown that the synthesis of metal nanoparticles using plant extracts, which is called "green synthesis", has some benefits such as low cost and low toxic effects to produce the large-scale metal nanoparticles [2-4].

Copper (Cu) is one of the most useful elements in medical science because of its numerous anti-inflammatory, anticancer, analgesic, and antimicrobial effects [5]. In recent years, it has been proven that, because of their high surface-to-volume ratio, copper nanoparticles (CuNPs) are extremely reactive and simply interact with other particles, leading to their wide range of biological activities [5-7].

Previous reviews on laboratory animals have demonstrated that liver is considered as the key target tissue of drug toxicity. Hence, assessing the function of this organ is among very important methods to determine drug toxicity [8]. Nowadays, one of the main criteria to determine the liver damage is measuring serum levels of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin [9-

11]. It has been reported that oral consumption of some medicinal agents may affect the hematological parameters and result in anemia, neutropenia, and thrombocytopenia; therefore, it is necessary to evaluate the effects of the novel medicinal drug by measuring the hematological parameters [10]. Accordingly, the present study was conducted to green synthesis of copper nanoparticles from *Capparis spinosa* fruit extract and evaluate their effects on liver function and hematological parameters in mice.

2. Materials and Methods

2.1. Green synthesis of copper nanoparticles

Fruits of *C. Espinosa* were collected from the rural areas in western Iran and then were extracted by percolation procedure by means of methanol (80%) for three days in room temperature [12]. In the next step, the green synthesis of CuNPs was performed according to the method described elsewhere. Briefly, 75 ml of the obtained extract was added to 100 ml 0.01 M copper sulfate solution. After stirring, it was kept at 60℃ for one day. In the next step, to remove all impurities, it was centrifuged twice at the 12,000 rpm for 20 min. Nanoparticles start to deposit when the color of the solution changed from green to amber yellow. The synthesized nanoparticles were heat treated in the oven at 60℃ for further analyses.

2.1.1. UV-Vis spectroscopy analysis

Transformation of the copper ions to copper nanoparticles was approved by the surface plasmon resonance (SPR) of the copper nanoparticles. For this purpose, 0.3 ml of the specimens was diluted with 3 ml of normal saline and studied via UV-Vis spectrum analysis by means of a spectrophotometer device (JENWAY 6405) in the range of 300-700 nm [13].

2.1.2. Fourier transform infrared spectroscopy

After pouring and mixing the obtained samples and potassium bromide (KBr) granules together with the ratio of 1 to 100 (1/100 ratio) and compacting them into tablets, FTIR (model Nicolet32) analysis was carried out in the range of 400-4000 and with the resolution of 1-4cm [14].

2.1.3. Scanning electron microscope (SEM)

To obtain the characteristics of synthesized nanoparticles, electron microscopy (Mira3, Made in Czech) with 15 kv, magnification of 10x, and resolution of 1 nm was performed.

2.2 Animals and Study design

A total of 32 male BALB/c mice weighing 25-30 were provided from the Tehran Pasteur Institute and kept with light-dark cycles (12:12-h). The room temperature was 22±2℃ and the mice had ad libitum access to water and food. They were placed under laboratory conditions 30 min before the start of the experiment. The ethical approval required for this study (No. 2018/A-10-1540-3) was issued by the Ethics Committee of Lorestan University of Medical Sciences, Lorestan, Iran. Overall, mice tested in this study were assigned to the following four groups:

Group i: received normal saline orally for 14 days;
Group ii: received the *CuNPs* at the concentration of 1000 µg/kg orally for 14 days;
Group iii: received the *CuNPs* at the concentration of 2000 µg/kg orally for 14 days;
Group iv: received the *CuNPs* at the concentration of 5000 µg/kg orally for 14 days;

2.3. Sample collection

On day 15 of the experiments, the mice were anesthetized by Ketamine-Xylazine followed by collecting blood samples from each mouse after opening the heart. Collected blood samples were put into tubes with or without anticoagulant to process their clot and then their sera were separated by centrifugation at 5000g for 10 min.

2.4. Evaluation of the serum liver enzymes

In this study, to determine the hepatoprotective effects of CuNPs, different clinical chemistry parameters related to liver function such as AST, ALT, ALP, and bilirubin (direct and total) were assayed by commercial diagnostics kits (Roche, Germany) [10, 15].

2.5. Hematological Parameters

To assess the effects of CuNPs on hematological studies, total collected blood was put into tubes containing ethylenediaminetetraacetic acid (EDTA). Next, hematological parameters including hemoglobin, hematocrit, white blood cell counts, red blood cell counts, and platelet counts were measured by Sysmex (KX-21, Japan).

2.6. Statistical analysis

SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the obtained data. One-way ANOVA test and Tukey's post-hoc test were performed to assess the difference between experimental groups. Also, $P<0.05$ was considered as statistically significant.

3. Results

3.1. UV-Vis spectrum analysis

The obtained results showed that the highest peak of the synthesized CuNPs was found in the wavelength of 414 nm. The characteristic of the resonance band of the surface plasmon happened for CuNPs at the wavelength of 414 nm (Fig 1).

3.2. FTIR analysis

The obtained findings of FTIR revealed that the biomolecules in the extract decreased the copper sulfate solution. The bands at 3380, 2928, 1741, 1604, 1400, 1050, and 1271 were indexed to the O-H stretching of alcohol and phenol, C-H stretching of the aliphatic group, C=O stretching of ester carbonyl, C=C stretching of the aromatic ring, and C-O stretching of ester, respectively (Fig 2).

3.3. SEM analysis

Following the approval of the synthesized nanoparticles through color modification and Vis-UV and FTIR, the characterization of nanoparticles was determined with SEM. As shown in Fig. 1, the synthesized copper nanoparticles represent the spherical morphology whereas the size of the particles was recorded to be between 17 and 41 nm (Fig. 3).

3.4. Hepatoprotective effects of CuNPs

Table 1 shows the results of hepatoprotective effects on serum biochemical parameters in mice receiving CuNPs at the doses of 1000, 2000, and 5000 µg/kg for 14 days. As can be seen, although these parameters increased with increasing dosage of extract, there is no statistically significant difference ($p> 0.05$) between oral administrations of CuNPs at the doses of 1000, 2000, and 5000 g/kg and control group.

3.5. Effect on hematological parameters

As shown in Table 2, following the oral administrations of CuNPs at the employed doses of at the doses of 1000, 2000, and 5000 µg/kg for 14 days, there was no significant difference ($p<0.05$) between hematological parameters compared with the control group.

4. Discussion

In recent years, studies in the field of nanotechnology demonstrated that physical and chemical procedures to create the nanoparticles, despite having exceptional biological activities, due to having some restrictions like toxicity, are switched by a number of new methods such as green synthesis [1]. Today, considering that the nanoparticles are broadly used by a large proportion of world populations to treat some diseases, it is of high necessity to measure the toxicity of these products by different methods. Since recent studies have shown that CuNPs possess a wide range of biological activities [16], we decided to evaluate its toxicity and hepatoprotective effects in mice model.

Recent studies have shown that liver enzyme measurement is one of the key diagnostic tests to evaluate the liver function and also inflammations and damage such as hepatitis and cirrhosis [8, 10]. CuNPs, because of their high surface-to-volume ratio are extremely reactive and simply interact with other particles. Therefore, they have numerous biological activities [17, 18]. It has been proven that some medications can cause irreparable complications through reducing white and red blood cells and the number of blood platelets; thus, it is necessary to evaluate the effects of the novel medicinal drug by measuring the hematological parameters [19].

Based on the results of the present study, after oral administration of mice with CuNPs at the doses of 1000, 2000, and 5000 µg/kg for 14 days, although some parameters increased with increasing dosage of extract, there was no statistically significant difference ($p> 0.05$) between oral administrations of CuNPs at these doses and control group. Moreover, no statistically significant difference ($p<0.05$) was observed in hematological parameters between mice treated with CuNPs and the control group.

Considering the study of hepatoprotective effects of nanoparticles and similar to our findings, Zhang et al (2018) have demonstrated that silver nanoparticles synthesized using *Rhizophora apiculata* were effective in protecting the liver from harms induced by carbon tetrachloride [20]. Kalirajan et al (2014) have reported that increased enzymatic levels of AST, ALT, ALP, and Bilirubin by CCl4 were returned to normal when treated with silver nanoparticles synthesized using fruit extract of *Embilica officinalis*; indicating potent hepatoprotective effects of these NPs [21]. In the study conducted by Eftekhari et al (2017), quercetin nanoparticles showed remarkable hepatoprotective activity via decreasing levels of AST, ALT, and ALP [22]. Ghosh et al (2016) also recently have shown that gold

nanoparticle synthesized by *Trigonella foenum* extract significantly normalized the increased enzymatic levels of AST, ALT, ALP, and Bilirubin induced by CCl4; which indicated hepatoprotective potential of these NPs [23]. In the present study, we applied *C. spinosa* to facilitate the synthesis of CuNPs. Next, we synthesized CuNPs with spherical and size ranging from 17 and 41 nm. To date, a wide range of plants such as *Syzygium aromaticum*, *Nerium oleander*, *Citrus medica Linn. (Idilimbu)*, *Capparis zeylanica*, *Gloriosa superba L.*, and *Vitis vinifera* have been applied in the biosynthesis of CuNP. However, identifying the plant's capacity as biological material for the synthesis of nanoparticles in full detail requires more investigations [5, 6, 17, 24, 25-27].

Conclusion

The findings revealed that copper nanoparticles biosynthesized from aqueous extract of *C. spinosa* fruit have no toxic effect on the liver of the studied mice; as well as no significant toxicity was observed on hematological parameters in mice. However, more studies need to be done for evaluation of hepatoprotective effect of CuNPs.

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References

1. Boisseau P, Loubaton B. Nanomedicine, nanotechnology in medicine. Comptes Rendus Physique 2011; 12: 62-6.
2. Mano Priya M, Karunai Selvia B, John Paul JA. Green synthesis of silver nanoparticles from the leaf extracts of *Euphorbia hirta* and *Nerium indicum*. Digest J Nanomat Biostruct 2011; 6: 869 -77.
3. Khatami M, Pourseyedi S. *Phoenix dactylifera* (date palm) pit aqueous extract mediated novel route for synthesis highstable silver nanoparticles with high antifungal and antibacterial activity. IET Nanobiotechnol 2015; 9:184-90.
4. Khatami M, Amini E, Amini A, Mortazavi SM, Kishani Farahani Z, Heli H. Biosynthesis of silver nanoparticles using pine pollen and evaluation of the antifungal efficiency. Iran J Biotechnol 2017; 15: 1–7
5. Saranyaadevi K, Subha V, Ernest Ravindran Rs, Renganathan S. Synthesis and caharacterization of copper nanoparticle using *Capparis zeylanica* leaf extract. Int J Chem Tech Res 2014; 6: 4533-41.
6. Naika HR, Lingaraju K, Manjunath K, Kumar D, Nagaraju G, Suresh D, Nagabhushana H. Green synthesis of CuO nanoparticles using *Gloriosa superba* L. extract and their antibacterial activity. J Taibah Uni Sci 2015; 9: 7-12.
7. Angrasan J, Subbaiya R. Biosynthesis of copper nanoparticles by Vitis vinifera leaf aqueous extract and its antibacterial activity. Int J Curr Microbiol Appl Sci 2014; 3: 768-74.
8. Mahmoudvand H, Fallahi S, Mahmoudvand H, Shakibaie M, Harandi MF, Dezaki ES. Efficacy of *Myrtus communis* L. to Inactivate the Hydatid Cyst Protoscoleces. J Invest Surg. 2016; 29(3):137-43.
9. Fallahi S, Beyranvand M, Mahmoudvand H, Nayebzadeh H, Kheirandish F, Jahanbakhsh S. Chemical composition, acute and subacute toxicity of *Satureja khuzestanica* essential oil in mice. J Res Pharm. 2017; 21: 515-521.
10. Mahmoudvand H, Tavakoli Oliaei R, Mirbadie SR3, Kheirandish F, Tavakoli Kareshk A, Ezatpour B, et al. Efficacy and Safety of *Bunium Persicum* (Boiss) to Inactivate Protoscoleces during Hydatid Cyst Operations. Surg Infect (Larchmt). 2016;17:713-719.

11. Mahmoudvand H, Mahmoudvand H, Oliaee RT, Kareshk AT, Mirbadie SR, Aflatoonian MR. In vitro Protoscolicidal Effects of *Cinnamomum zeylanicum* Essential Oil and Its Toxicity in Mice. Pharmacogn Mag. 2017;13 Suppl 3: S652-S657.

12. Ebrahimi K, Shiravand M, Mahmoudvand H. Biosynthesis of copper nanoparticles using aqueous extract of *Capparis spinosa* fruit and investigation of its antibacterial activity. Marmara Pharm J. 2017; 21: 866-871.

13. Khatami M, Amini E, Amini A, Mortazavi SM, Kishani Farahani Z, et al. Biosynthesis of silver nanoparticles using pine pollen and evaluation of antifungal efficiency. Iran J Biotechnol 2017; 15: 1-7.

Clinical biochemistry parameters	AST (U/L)	ALT (U/L)	ALP (U/L)	TB

14. Soltani Nejad M, Khatami M, Shahidi Bonjar GH. Extracellular synthesis gold nanotriangles using biomass of Streptomyces microflavus. IET Nanobiotechnol 2016; 10: 33-8.

15. Mahmoudvand H, Kheirandish F, Dezaki ES, Shamsaddini S, Harandi MF. Chemical composition, efficacy and safety of *Pistacia vera* (var. Fandoghi) to inactivate protoscoleces during hydatid cyst surgery. Biomed Pharmacother. 2016;82:393-8.

16. Murthy SK. Nanoparticles in modern medicine: State of the art and future challenges.Int J Nanomedicine. 2007; 2: 129-41.

17. Gopinath M, Subbaiya R, Selvam MM, Suresh D. Synthesis of copper nanoparticles from *Nerium oleander* leaf aqueous extract and its antibacterial activity. Int J Curr Microbiol Appl Sci 2014; 3: 814-8. 21.

18. Katha U, and Gajera H. Synthesis of copper nanoparticles by two different methods and size comparison. Int J Pharm Bio Sci 2014; 5: 533-40

19. Muriithi NJ, Maina GS, Mugendi NM, Maina MB, Kiambi MJ, et al.(2015) Determination of Hematological Effects of Methanolic Leaf Extract of *S. incanum* in Normal Mice. Pharm Anal Acta 2015; 6: 429. doi:10.4172/21532435.1000429

20. Zhang H, Jacob JA, Jiang Z, Xu S, Sun K, Zhong Z, Varadharaju N, Shanmugam A. Hepatoprotective effect of silver nanoparticles synthesized using aqueous leaf extract of Rhizophora apiculata. 2019;14: 3517—3524.

21. Bhuvaneswari R, Chidambaranathan N, Jegatheesan K. Hepatoprotective effect of Embilica officinalis and its silver nanoparticles against ccl4 induced hepatotoxicity in Wistar albino rats. Digest Journal of Nanomaterials and Biostructures 2014; 9(1): 223-235.

22. Eftekhari A, Ahmadian E, Panahi-Azar V, Hosseini H, Tabibiazar M, Maleki Dizaj S. Hepatoprotective and free radical scavenging actions of quercetin nanoparticles on aflatoxin B1-induced liver damage: in vitro/in vivo studies. Artificial Cells, Nanomedicine, and Biotechnology 2018; 46(2): 411-420.

23. Ghosh S, Gomes A. Hepatoprotective efficacy of gold nanoparticle synthesized by green method using Trigonella foenum-graecum seed extract. Transl Med (Sunnyvale) 2016, 6:4

24. Subhankari I, Nayak P. Synthesis of copper nanoparticles using *Syzygium aromaticum* (Cloves) aqueous extract by using green chemistry. World J Nano Sci Technol 2013; 2: 14-7.

25. Shende S, Ingle AP, Gade A, Rai M. Green synthesis of copper nanoparticles by *Citrus medica* Linn.(Idilimbu) juice and its antimicrobial activity. World J Microbiol Biotechnol 2015; 31: 865-73.

26. Janakiraman M. Protective efficacy of silver nanoparticles synthesized from *Silymarin* on *cisplatin* induced renal oxidative stress in albino rat. Inter J Appl Pharm. 2018; 10: 110-16.

27. Murugesan S, Bhuvaneswari S, Sivamurugan V. Green synthesis, characterization of silver nanoparticles of a marine red alga *Spyridia fusiformis* and their antibacterial activity. Inter J Pharm Pharm Sci. 2017; 9: 192-7.

Table 1. Effects of CuNPs on serum liver enzymes after two weeks administration in mice. The results were given as mean ± standard deviation (SD)

	Control	130.6±8.15	38.3±4.31	132.3±8.15	0.12±0.03
	Cu NPs (1000 µg/kg)	142.4±6.15	40.3±3.15	139.6±6.5	0.15±0.05
	Cu NPs (2000 µg/kg)	138.8±5.51	39.3±2.51	142.3±5.01	0.14±0.04
	Cu NPs (5000 µg/kg)	144.3±8.15	42.7±4.36	144.6±6.15	0.17±0.05

BUN, Blood urea nitrogen; Cr, creatinine; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; TB, total bilirubin

Table 2. Hematology parameters in whole blood of mice following oral administration of CuNPs for two weeks. The results were given as mean ± standard deviation (SD)

Parameters	*C. longa* essential (µg/kg)			Control
	1000	2000	5000	
RBC ($\times 10^6/\mu L$)	3.7 ± 0.13	3.2 ± 0.25	2.9 ± 0.41	3.4 ± 0.3
HGB (g/dL)	11.2 ± 0.6	10.5 ± 1.15	10.1 ± 0.6	11.3 ± 0.45
Hct (%)	33.7± 3.1	32.12 ± 2.15	30.4 ± 2.51	32.6 ± 2.18
WBC ($\times 10^3/\mu L$)	3.3 ± 0.45	2.7 ± 0.26	3.1 ± 0.25	2.8 ± 0.2
PLT ($\times 10^3/\mu L$)	187 ± 15	193 ± 13	175 ± 11	184 ± 17

RBC, red blood cell; HGB, hemoglobin; Hct, hematocrit; WBC, white blood cell; PLT, platelet

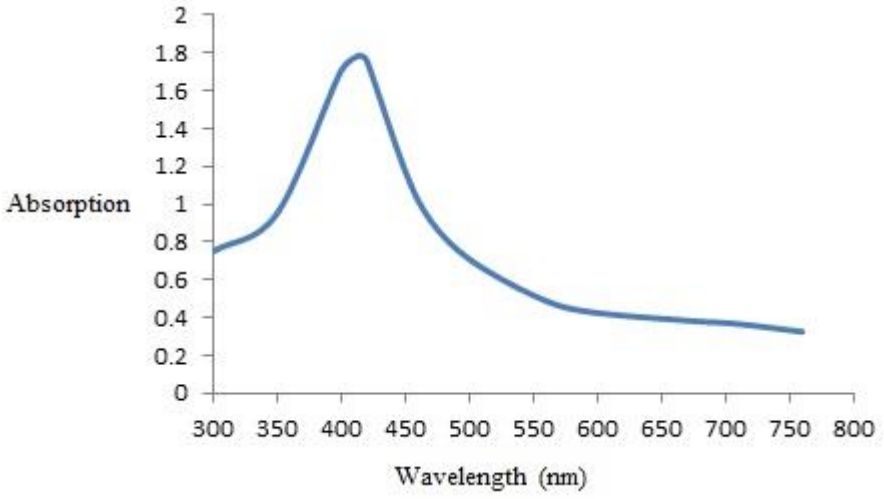

Figure 1. The absorption spectrum of synthesized copper nanoparticles.

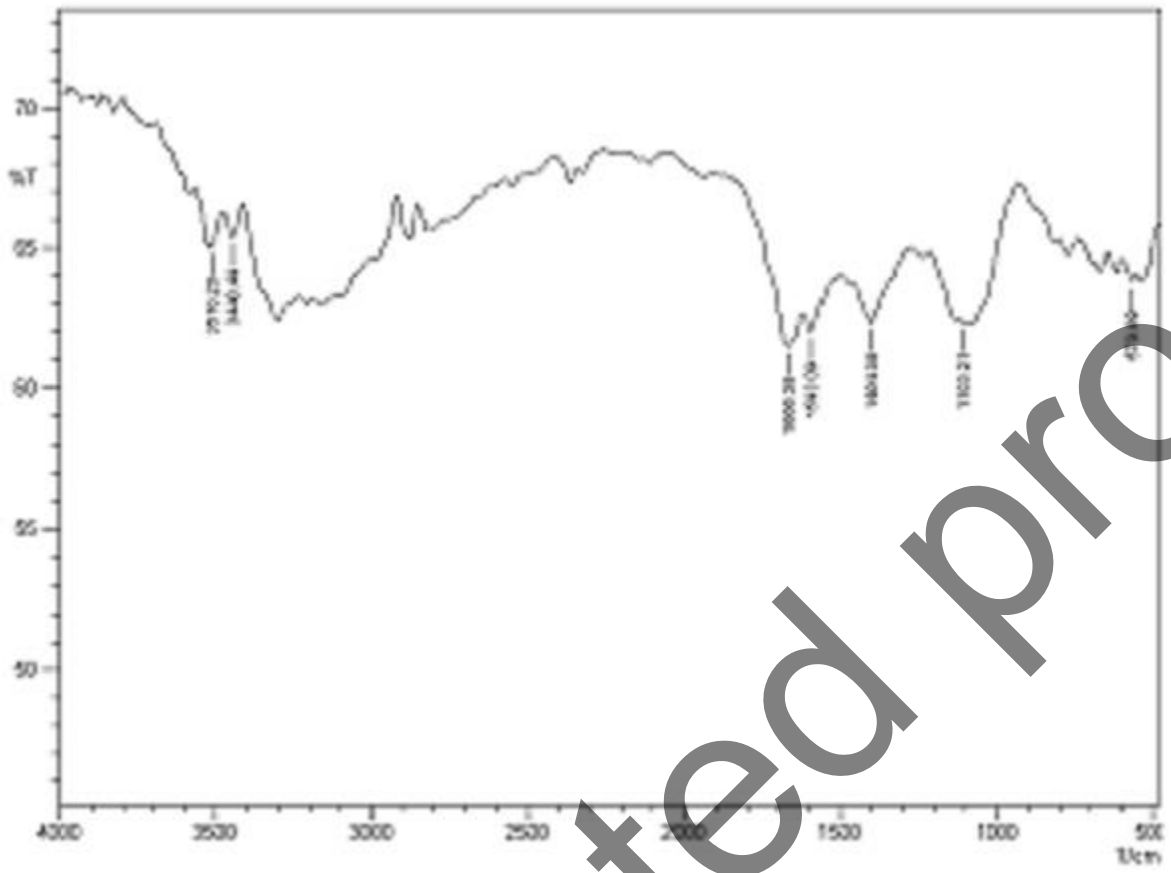

Figure 2. The FTIR spectrum of synthesized copper nanoparticles.

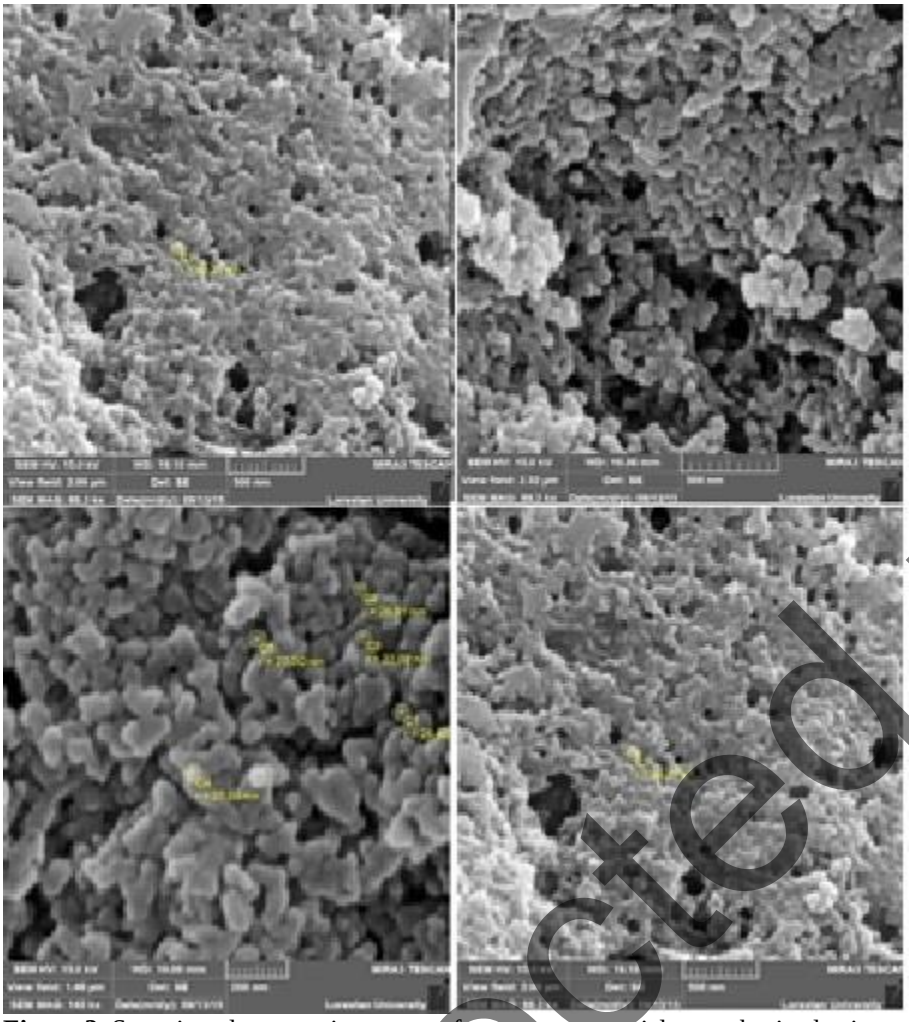

Figure 3. Scanning electron microscope of copper nanoparticles synthesized using aqueous extract of *Capparis spinosa* fruit