Journal of Materials Science & Nanotechnology
Volume 7 | Issue 1
ISSN: 2348-9812

Research Article Open Access

Copper Oxide Nano-Hydrogel Composite and their Toxicology Studies: A Green Chemistry Approach

Ezealisiji KM*[1], Siwe-Noundou[2] and Krause RWM[2]

[1]Department of Pharmaceutical and Medicinal Chemistry, University of Port Harcourt, Nigeria
[2]Department of Chemistry, Rhodes University, South Africa

***Corresponding author:** Ezealisiji KM, Department of Pharmaceutical and Medicinal Chemistry, University of Port Harcourt, Nigeria, Tel: +234-805-646-3129, E-mail: Kenneth.ezealisiji@uniport.edu.ng

Citation: Ezealisiji KM, Siwe-Noundou, Krause RWM (2019) Copper Oxide Nano-Hydrogel Composite and their Toxicology Studies: A Green Chemistry Approach. J Mater Sci Nanotechnol 7(1): 105

Received Date: March 23, 2019 **Accepted Date:** May 13, 2019 **Published Date:** May 15, 2019

Abstract

The increasing demand for skin care products formulated with metal oxide nanoparticles such as Zinc oxide, Titanium oxide and Copper oxide nanoparticles calls for toxicological safety concerns. Phytochemical assisted synthesis of Copper oxide nanoparticles (CuONPs) has been achieved by a simple, one-pot chemical procedure using polyphenol-rich aqueous extract of *Solanum torvum* L. as a reducing and capping agent. The end product of the reaction (CuONPs) was confirmed by Ultraviolet-visible (UV-vis) spectroscopy, Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Fourier Transform Infrared spectroscopy, X-ray diffraction (XRD) and Dynamic light scattering technique (DLS). TEM and XRD studies showed that the formed copper oxide nanoparticles were crystalline and monoclinic in shape with an average particle size of 32±0.25 and 22.76 nm respectively. The present study also investigated the dose and time-dependent effects of CuONP-Hydrogel composite applied on rat skin for 7, 14, or 28 days; with carbohydrate polymer based hydrogel as a vehicle control. Blood samples were obtained and hematological indices were measured. CuONP-Hydrogel composite increased ($p < 0.05$) the White blood cells (WBC) count at both lower and higher doses; it produced a significant time dependent effect on WBC. The Red Blood Cells (RBC) count and hematocrit were also reduced. Platelet count were significantly ($p < 0.05$) increased in all treatment groups. This research therefore affirms that chronic use of CuONPs in dermatological preparations could affect hematological functions in rats. As such, care should be taken in human use.

Keywords: Phytochemical; Polyphenol; Hematological; Nanoparticles; Hydrogel

Introduction

The use of Copper oxide nanoparticles (CuONPs) in a wide range of applications such as Optical sensors, Catalytic, Electronics, Superconductors and Biomedical field have attracted lots of interest in recent time [1-6]. The importance of Copper oxide nanoparticles in biomedical fields has also gained significant attention and these include their uses in footwear and blankets in the hospitals to prevent nosocomial infections [7-8]. It can also be used as an antifungal and antimicrobial agent in several health related applications ranging from cosmetics to pesticidal preparations [9]. General method of fabrication of Copper oxide nanoparticles includes chemical synthesis, which may lead to deposition of some toxic bye products on the surface of the metal nanoparticles but recent development involving Green synthesis using plant extracts such as *Lemon grass*, *Euphorbia tirualli, Gloriosa superba* and *Cinnamomum camphora* have been reported [10-13]. Nanoparticles synthesized from the above method exhibit varying bioactivities including anticancer, anti-inflammatory, anti-microbial, and wound healing [14-16]. *Solanum torvum* L. is a species of flowering plant in the family of Solanaceae. It is indigenous to Africa and Asian countries such as India and Pakistan. The fruits are edible with slightly bitter taste. The leaves are rich in Antioxidants, Polyphenols and alkaloids and have been used ethno medicinally in the management of asthma , cough, arthritis, infertility, cholera, sexually transmitted disease and malaria [17-20]. In recent times, the use of Copper oxide nanoparticles in cosmetics and biomedical application that encourage contact of metal nanoparticles with the skin has attracted toxicological concerns since it has not taken into consideration of the use of penetration enhancers such as hydrogels as drug delivery vehicle. Synthesis of CuONPs from *Solanum torvum* has not been reported in past literature and toxicology effects of CuONP-Hydrogel composite in rats have not been documented.Hence present work has been undertaken to synthesize CuONPs and to investigate the dose and time-dependent effects of CuONP-Hydrogel composite applied on rat skin for 7, 14, or 28 days. The novelty of this study lies on the use of skin penetration enhancer such as hydrogel as nanocarrier across the skin. Penetration enhancers are known to modify the architectural integrity of the skin raising the question on what happens to nanoparticles (formulation in hydrogel) of slightly higher than normal size when they come into contact with the intact skin. Present study is directed towards bridging this knowledge gap.

Experimental Section

Materials

Copper sulphate, Ethanol and deionized water were of analytical grade and product of Merck, Germany, and Oxoid Hampshire UK. Reagents were used as such without further purification. Fresh leaves of *Solanum torvum* L were harvested from the Pharmacognosy garden of the University of Port Harcourt, Nigeria and were further identified by Dr. Ekeke Chimezie of the Department of plant science and Biotechnology Unit of University of Port Harcourt. The voucher specimen was deposited in the herbarium.

Instrumentation

The surface topography and particle size of the CuONPs were determined by transmission electron microscopy (TEM) on a ZEISS LIBRA 120 KV-UK, at varying magnification. Bruker d8, Japan; advanced X-ray difractometer, using CuKα radiation (λ=1.5406 ä) 40 Kv, 2 θ/θ scanning mode were employed to analyze the X-ray diffraction (XRD) parttern of the biosynthesized CuONPs. This high thorough-put study was carried out at the Department of Chemistry, Rhodes University Nanotechnology Centre. Data was obtained for the 2θ range of 10 – 120 degree in step proceeding of 0.0206 degree. Fourier transform infrared (FTIR) spectroscopy was performed using Shimadzu 84005, Japan. Microscopic analysis was carried out using Scanning Electron Microscopy (SEM) on a TESCAN VEGA LMU, Germany. Formation of CuONPs using S.torvum leaf extract was monitored by visual colour change, while Surface Plasmon Resonance (SPR) of CuONPs was characterized using UV-Vis spectrophotometer (Perkin Elmer Lambda 35 Uv-Vis spectrophotometer, Germany. Dynamic light scattering (DLS) and Zeta potential of the synthesized nanoparticles was analyzed to know the average size and stability of particles using (DLS-Nano 2s model, UK).

Preparation of Leaf Extract

The leaves of *S.torvum* L were carefully rinsed with tap water to remove dirt particles and other impurities. Ten grams (10 g) of dried leaves were ground, poured into an Erlenmeyer flask and boiled with distilled water for 30 min. The hot aqueous extract was filtered through 0.45 µm sintered glass funnel and kept in a refrigerator until use.

Green Synthesis of CuONPs

For the preparation of CuONPs, 250ml of 1.5 mM of copper sulphate aqueous solution was carefully transferred into an Erlenmeyer flask holding 250ml of extract of S.torvum leaves. The flask was incubated in the dark at 37 °C for six hours with constant stirring. A control setup was also maintained without the leaf extract. The formation of CuONPs was confirmed by colour change from yellowish brown to dark brown.

CuONano-Hydrogel Composite Preparation

A 1.5 kg of Ipomoea batatas (sweet potatoes) was purchased from the local market in Port Harcourt, Nigeria. Peeling, washing, wet milling and further washing with water was ensured to remove the starch. The resultant cellulosic fibre was dried in a hot air oven, pulverized and passed through a 180 µm stainless steel sieve. The product was treated with sodium hypochlorite (3.5% w/v), washed in ethanol (95% w/v) and basified to get the hydrogel. A 0.5 g quantity of CuONPs was then infused into 95 g of the hydrogel. Both were triturated to homogeneity in a porcelain mortar and the amount made up to 100 g of the preparation with the hydrogel. Further trituration to ensure homogeneity was done. A similar step was taken to formulate the 1.0% w/w preparation.

Animals

Male and female Wister rats weighing 200-250 g were sourced from the animal house of the Department of Pharmacology, University of Port Harcourt. The rats were adequately fed with rodent feed and allowed free access to table water ad Libitum. The animals were housed in an animal room maintained at 24.5 ± 0.5 °C and 52.8±4% relative humidity with an alternating 12:12-hour light-dark cycle. All animal experimental protocol herein was reviewed and vetted by the University of Port Harcourt use of animal Ethics Committee.

Particle Size Analysis

Convectional image processors such as Image J software were employed in the particle size analysis of the biosynthesized copper oxide nanoparticles. The Image J software is a multifunctional image processing entity with an inbuilt Java analytical tool. The Java enabled programe is used in the analysis of sub-micron images. In the present study, the size distribution and average particle size were analyzed using the Image J software. The crystallographic toolbox (Crys TBox) was also used for the XRD analysis.

Sub-Chronic Toxicity Study

The animals were randomly divided into four groups of six rats each. Different doses of the Bio-synthesized copper oxide nano-hydrogel composite were applied to the rat skin to investigate the toxicity of CuONPs on cumulative dermal exposure. A 4 cm x

4 cm area on the dorsal region of each rat was depilated once weekly. CuONP-hydrogel composite was applied to 10% of the total body surface area (4 cm x 4 cm). The area under application was covered with sterile occlusive dressing to prevent other rats from licking it up. The procedure was repeated every day for 28 days. Group 1 (normal control) animals were treated with distilled water for 28 days. Group 2 was administered carbohydrate-polymer hydrogel only as a vehicle control group. Group 3 and 4 were treated with copper oxide nano-hydrogel composite at doses of 0.5% w/w and 1.0% w/w, respectively. At the end of 28 days, the animals were sacrificed (euthanasia) and blood sample taken for analysis.

Elemental Plasma Copper Level Analysis

A Vario 6 Graphite furnace (Analytik Jena, Germany) Spectrometer was employed for elemental analysis of serum copper level in rats at the wavelength of 324.8 nm, slit width of 0.8 nm and Atomization temperature of 1800-1900 °C. Standard calibration curves were prepared using a linear curve. Data analysis was also carried out using SPSS data editor 16.0 and Microsoft office Excel Version 2007.

Results and Discussion

The phyto-extract assisted fabrication of metal oxide nanoparticle with other bioreductants affords a simple and cost effective synthetic step [21]. Phytochemicals are known not only to reduce metal ions but are also responsible for capping and stabilization of these nanoparticles. In the current study, leaf extract of *Solanum torvum* has been employed for the green synthesis of CuONPs followed by detailed toxicological study of their effect on haematological index in rats after dermal application for 28 days.

Physicochemical Characterization of CuONPs

Ultraviolet-Visible Spectroscopy: To determine the development of metal oxide nanoparticles, the matter of first importance is the visual or organoleptic characterization of the color change during the course of synthesis. Plasmon bands were observed from sample isolated at the end of the synthesis. The preparation of CuONPs was identified by appearance of brown to dark brown color. The mechanisms of the synthetic reaction follow a stepwise chemical interaction between copper (II) sulphate pentahydrate and sodium hydroxide to give copper hydroxide, which further reacts with Phyto-polyphenols to yield copper (I) oxide. Subsequent aggregation of copper (I) oxide gave CuO nanoparticles as shown below.

$CuSO_4.5H_2O + 2NaOH \rightarrow Na_2SO_4 + Cu(OH)_2 + 5H_2O$
$2Cu(OH)_2 + (\text{Phyto-Polyphenols}) \rightarrow Cu_2O + \text{other end products}$
$Cu_2O + OH^- + H_2O \rightarrow (Cu[OH]_2) + CuOH$
$2CuOH + Cu_2O + H_2O$
$4CuOH + O_2 \rightarrow 4CuO + 2H_2O$

The partly spherical CuO nanoparticles formed are stabilized and capped by phytochemicals, which imparts stability to the synthesized product. Analysis of samples using UV- vis spectrophotometer between the scan range of 180-700 nm gave a surface Plasmon resonance (SPR) spectra with fairly broad peak at 280 nm corresponding to copper oxide nanoparticles (Figure 1). A result similar to this finding was confirmed from copper oxide nanoparticles fabricated with the aid of *Syzygium alternifolium* stem bark aqueous extract [22].

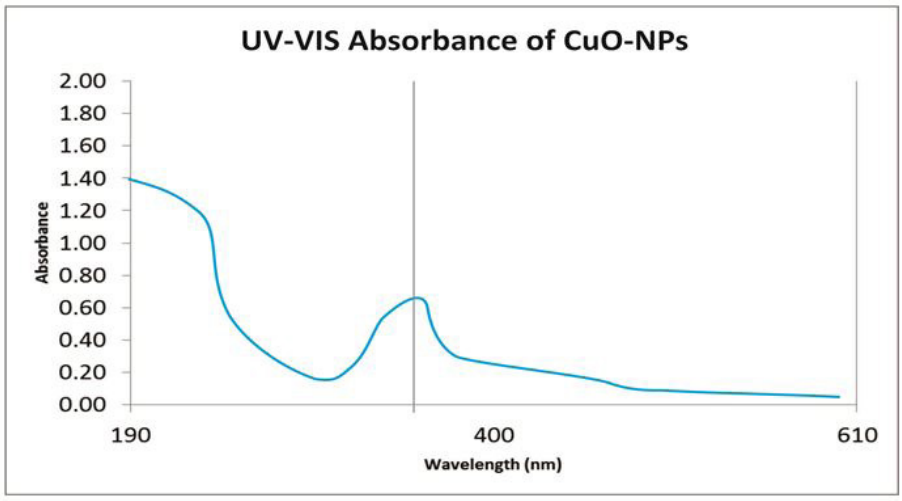


Figure 1: UV-Vis Absorbance of CuO-NPs

FTIR analysis of *Solanum torvum* aqueous extract: The FT-IR spectra of Solanium torvum extract are presented in Figure 2. Strong absorption peaks of *Solanum torvum* leaf extract were observed at 3362, 2920, 1652, 1389, 1068, and 620 cm^{-1} due to the

presence of N-H stretching vibration of amine groups and possible -OH groups of Polyphenols, N-H of primary amide groups and C-H stretching [23]. The peaks of 1652 and 1389 cm^{-1} corresponds to amide-I stretching which strongly supports the presence of amide or Polyphenol functional group of *Solanum torvum*. These confirmed functional constituent groups are believed to have acted as reducing and capping agents for the formation of CuONPs, the evidence is based on non appearance of new bands as seen under the three different FTIR spectra (Figure 2).

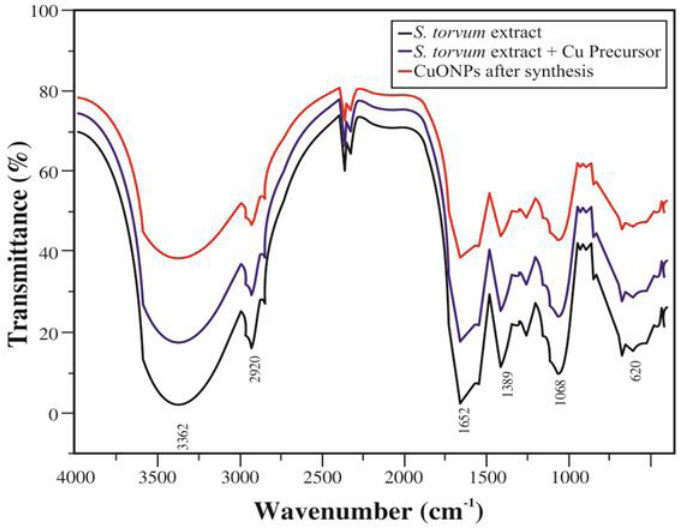
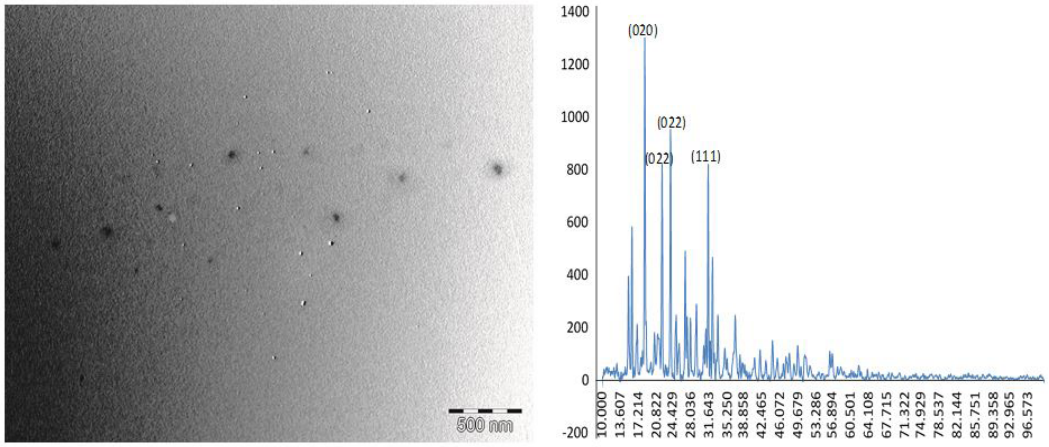


Figure 2: FT-IR of leaf extract of S.torvum, extract + Cu precursor as well as CuONPs after synthesis

TEM, SEM and XRD analysis: Measurements obtained from Transmission electron microscopy (TEM) revealed particle size and morphology of the synthesized CuONPs. Synthesized CuONPs were spherical in shape, non-clustering, well dispersed with size range between 28 and 45 nm with a mean particle size 32±0.25 nm (Figure 3a). Scanning electron microscopy put through the particle morphology of the synthesized CuONPs. Synthesized nanoparticle was justified spherical in shape with few non spherical crystalline structures. All the X-Ray peaks are well consistent, corresponding accordingly with JCPDS card (Card no: 45 – 0967). Four prominent peaks at 2 θ values of 18.406°, 22.102°, 23.672°, and 30.902° corresponding to, (020), (022), (022), and (111) integer on the `hkl` planes respectively were observed. This may be recorded as bands for face-centred and crystalline particulate matter which is spherical in nature (Figure 3b). Present result showed a remarkable correlation with matched data obtained from International Centre for Diffraction Data (ICDD). Using Debby-Scherrer equation: $D = \frac{K\lambda}{{}_{\beta} Cos\,\theta}$

(where D is the thickness of the nanocrystal, K is a constant; λ is the Bragg`s angle 2θ).

The average particle size of the CuONPs was calculated to be 22.76 nm at an operational Optimum Bragg reflection obtained at 2θ of 18.204°, which is confirmed by the TEM study as well.



(Figure 3a) (Figure 3b)

Figure 3a; b: TEM image and XRD image of biosynthesized CuONPs

DLS and Zeta Potential: The calculated average size distribution of the biosynthesized Copper oxide nanoparticle is 396±1.64 nm from the DLS study (Figure 4). In general, it is expected that the size of CuONPs obtained from DLS could be bigger than those obtained from TEM and XRD. The increase in size could be due to the fact that the DLS measures the apparent size (hydrodynamic radius or diameter) of a particle as well as hydrodynamic layers that can be formed around hydrophobic

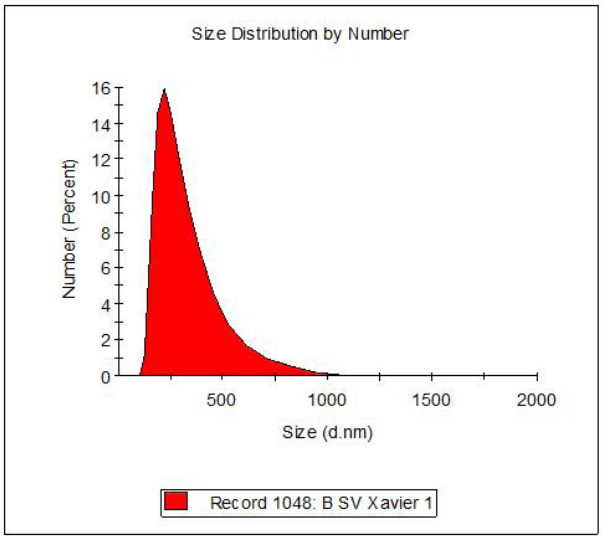


Figure 4: DLS Particle size distribution of synthesized CuONPs

particles. Also the above result could be attributed to the influence of solvation layer effect of electrolyte dispersion and restrictions in bond and rotation angles, leading to larger sizes in colloidal water media than the `geometric` sizes estimated by TEM and XRD. The synthesized nanoparticles are positively charged and moderately dispersed in the medium as the Zeta potential was found to be 48.3 mV.

Plasma Concentration of Copper: Elemental analysis using Atomic Absorption Spectroscopy (AAS) revealed that plasma Copper level in all rats treated with 0.5% w/w and 1.0% w/w CuONPs-hydrogel composite for 7, 14, and 28 days, showed a significant increase in dose and time dependent manner, $p < 0.05$, as compared to rats in the control as well as vehicle control group (Table 1). The moisturising and humectants properties of hydrogels were brought to bear in present work. The observed increase in plasma Copper concentration is as a result of high permeability profile through the skin when CuONPs were delivered through hydrogels as drug carrier.

Dose		Copper PPM	
	7 Days	14 Days	28 Days
Control	1.60±0.008	1.60±0.007	1.60±0.004
Veh. Cont.	1.40±0.001	1.60±0.001	1.30±0.002
0.5% w/w	5.62±0.002*	8.82±0.002*	10.44±0.004*
1.0% w/w	12.84±0.004*	18.68±0.002*	22.20±0.004*

*Significant at p<0.05, Data expressed as mean ± SEM.

*Veh. Cont = vehicle control

Table 1: Plasma level of Copper at different doses of Copper Oxide nanoparticle infused hydrogel in rats

Sub-Acute Toxicity Studies

Effect of Dermatological Application of CuONP-Hydrogel Composite on Haematological Indices of Rats: On analysis of whole blood, the packed cell volume and hematocrit level decreased significantly in all rats in the experimental groups exposed to 0.5% w/w and 1.0% w/w CuONPs-Hydrogel composite for 7, 14 and 28 days, when compared to control groups . In like manner, the haemoglobin levels of the rats in the experimental groups were significantly reduced (Table 2 and 3). CuONPs-Induced haemoglobin reduction was found to be significantly dose and time dependent.

Dose	PCV (%)			Hb (g/dL)		
	7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
Control	48±2.00	48±4.00	48±1.00	14.8±1.20	14.0±1.20	14.8±1.20
Veh. Cont	46±4.00	48±2.00	46±2.00	14.6±2.00	14.8±2.00	13.8±4.00
0.5% w/w	36±2.00	32±1.00	28±2.00	11.0±1.30	10.0±1.00	8.7±2.00
1.0% w/w	32±1.00	26±2.00	22±4.00	8.6±2.00	6.2±1.00	4.8±2.00

*Significant at p<0.05, Data expressed as mean ± SEM.

Table 2: Packed cell volume (PCV) and Hemoglobin level (Hb) following dermal administration of CuONPs-Hydrogel composite in rats

Dose	RBC (x10^{12}/L)			WBC (x10^{9}/L)		
	7 days	14 days	28 days	7 days	14 days	28 days
Control	6.8±0.02	6.8±0.02	6.8±0.02	4.2±0.02	4.2±0.01	4.2±0.02
Veh.cont	6.6±0.02	6.8±0.02	6.7±0.02	4.2±0.01	3.8±0.01	4.2±0.01
0.5% w/w	6.0±0.02	5.0±0.01	4.8±0.01	4.8±1.00	6.5±1.02	8.5±2.00
1.0% w/w	4.6±0.02	3.2±0.02	3.0±0.01	5.8±1.00	8.6±2.00	10.2±1.00

*Significant at $p < 0.05$, Data expressed as mean ± SEM.

Table 3: Red blood cell (RBC) and White blood cell (WBC) count following dermal administration of CuONPs-Hydrogel composite in rats

There was no change observed in platelet count in rats from all experimental groups after 7 days of administration of CuONPs but there was a significant increase in platelet count after 14 and 28 days. No significant changes were observed for the neutrophil count after 14 and 28 days respectively (Table 4).

Dose	Platelet count (x10^{9}/L)			Neutrophil (%)		
	7 days	14 days	28 days	7 days	14 days	28 days
Control	220±4.00	220±4.00	221±1.00	25±1.00	25±1.00	26±1.00
Veh.cont	221±1.00	220±4.00	220±4.00	26±1.00	25±1.00	25±1.00
0.5% w/w	230±3.00	290±2.00	320±1.00	30±1.00	34±2.00	40±2.00
1.0% w/w	250±2.00	300±2.00	360±1.00	32±2.00	42±1.00	48±2.00

*Significant at $p<0.05$, Data expressed as mean ± SEM.

Table 4: Platelet and Neutrophil count following dermal administration of CuONPs-Hydrogel composite in rats

There were no significant changes in the monocyte count of the rats in all experimental groups when compared to the control group. The lymphocyte count of rats in all experimental groups was found to be significantly elevated in dose and time dependent manner (Table 5).

Dose	Lymphocyte (%)			Monocyte (%)		
	7 days	14 days	28 days	7 days	14 days	28 days
Control	60±3.00	60±2.00	60±1.00	2±0.001	2±0.001	2±0.001
Veh.cont	60±3.00	60±3.00	60±2.00	2±0.002	2±0.001	2±0.001
0.5% w/w	68±2.00	72±1.00	78±2.00	2±0.001	2±0.001	2±0.001
1.0% w/w	75±1.00	80±1.00	86±2.00	3±0.200	3±0.002	3±0.002

*Significant at $p<0.05$, Data expressed as mean ± SEM.

Table 5: Lymphocyte and monocyte count following dermal administration of CuONPs-Hydrogel composite in rats

In present study, haematological parameters were measured up to 28 days after starting the exposure of different sub-lethal doses of Copper oxide Nano-hydrogel composite (0.5% w/w and 1.0% w/w). The mean PCV, Hb, RBC, WBC of CuONPs-Hydrogel composite exposed to sub-chronic treated rats are presented in (Table 2 and 3). The alterations observed in haematological parameters were significant ($p < 0.05$) compared to the control. Significant variation ($p < 0.05$) was also observed between the various haematological parameters with different concentrations of CuONPs-Hydrogel composite. Hematological profile is of immense importance in the studies of toxicological impact of toxicants on animal tissues. Various haematological parameters are often subjected to changes depending upon stress conditions.Reduction or rise in certain blood indices can be associated with the nature of toxicants. Decrease in haematological variables (PCV, Hb, and RBC) of exposed rats may be due to haemolysis and shrinkage of red blood cells by CuONPs resulting in significant decrease in hematocrit value which could result in anaemia. The observed reduction in RBC count was as a result of RBC breakdown and reduction in its formation. There could also be a decrease in hematopoiesis. Similar reduction in RBC was reported for Copper nanoparticle treated rats [24]. The PCV was significantly decreased with increasing toxicity of CuONPs after 28 days of exposure due to reduction in RBC. The observed increase in WBCs count occurred as a pathological response to changes in the internal chemistry of the animal. The WBCs are known to play a role during external invasion by foreign bodies or infection by stimulating the hemopoietic tissues and the immune system by producing antibodies and chemical defence against infection. Reduction in haemoglobin content of rats treated with CuONPs-Hydrogel could be attributed to reduction in haemoglobin synthesis. The general reduction in haematological indices of treated rats showed that physiological activities of the treated rats were grossly affected.

Conclusion

Phytochemical assisted fabrication of CuONPs has been successfully adapted in this research using aqueous leaf extracts of *Solanium torvum* L. Remarkable colour change justifies the formation of the metal nanoparticles. UV-Vis spectral characterization, TEM and XRD microscopy reveals the morphology of the fabricated nanoparticles as crystalline particulate

matter which is spherical in nature, with a size range of 22.76 nm 32±0.25nm. Mechanism of nanoparticles skin penetration is believed to be due to the modification and distortion of the skin architectural integrity by the hydrogel followed by pinocytocis. There is an observed significant increase in plasma Copper level in a dose and dependent manner. Toxicological studies carried out showed that haematological parameters were affected by dermatological exposure of CuO-Nano-Hydrogel composite in rats, this observation calls for CuONP toxicological concerns in human when used for a prolonged period in cosmetics.

References

1. Tian L, Ahmad F, Elliot S, Sonal P (2017) Size Controlled Copper (1) Oxide Nanoparticles Influence Sensitivity of Glucose Biosensor. Sensors 17: 1944.

2. Manoj BG, Anandrup G, Francois-X F, Tewodros Xiaoxi H, et al. (2016) Cu and Cu-Based Nanoparticles: Synthesis and Application in Catalysis. Chem Rev 116: 3722-811.

3. Sagandrevan S, Priya (2015) Electrical Properties of Copper Oxide Nanoparticles. J Nano Res 30: 1-8.

4. Seyed JD, Ramni K, Vahabodin G, Farideh P (2015) Synthesis of Copper(II) Oxide (CuO) Nanoparticles and its Applications as Gas Sensor. J Appl Biotechnol Rep 2: 329-32.

5. Mohammod A, Leong MK, Wong HL (2017) Green Synthesis of Copper Oxide (CuO) Nanoparticles using banana peel extract and their Photocatalytic activities. AIP Conf 1828.

6. Vinod VTP, Miroslav C (2013) Green Synthesis of Copper Oxide (CuO) Nanoparticles using gum Karaya as biotemplate and their anti bacterial application. Int J Nanomed 8: 86-92.

7. Azam A, Ahmed AS, Oves M, Khan M, Memic A (2012) Size-Dependent antimicrobial properties of CuO nanoparticles against Gram positive and negative bacterial strains. Int J Nanomed 7: 136-43.

8. Hadi SES, Hamid H, Vahid R, Seyed MJM, Mohammad M, et al. (2018) Promising Antimicrobial Effect of Copper Oxide Nanoparticles against Several Multidrug Resistant Uropathogen; Pharm Sci 24: 213-8.

9. Shaker AM, Zaki AH, Elham F, Abdel-Rahim, Khedr MH (2016) Novel CuO nanoparticles for pest management and pesticide photodegradtion. Adv Env Biol 10: 274-83.

10. Iravani S (2011) Green Synthesis of metal nanoparticles using plants. Green Chem 13: 2638-50.

11. Lee HJ, Lee G, Jang NR, Yum JH, Song JY, et al. (2011) Biological Synthesis of Copper nanoparticles using plant extract. Nanotechnol 128: 83-9.

12. Naika RH, Lingaraju K, Manjunath K, Kumar D, Nagaraju G, et al. (2015) Green Synthesis of Copper Oxide (CuO) Nanoparticles using Gloriosa superb L. extract and their antimicrobial activity. J Taibah Univ Sci 9: 7-12.

13. Huang J, Li Q, Sun D, Yinghua Lu, Yuanbo Su, et al. (2007) Biosynthesis of silver and gold nanoparticles by Novel sundried Cinnamomum camphora leaf. Nanotecnol 18: 105104.

14. Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M (2003) Extracellular biosynthesis of monodispersed gold nanoparticles by a novel extramophilic actinomycetes, Thermomonospora sp. Langmur 19: 3550-3.

15. Rehana D, Mahendirrana D, kumar S, Rahiman AK (2017) Evaluation of antioxidant and anti-cancer activity of Copper Oxide nanoparticles synthesized using medicinally important plant extract. Biomed Pharmacother 89: 1067-77.

16. Yugandhar P, Vasavi T, Devi PUM, Savithramma N (2017) Bioinspired green synthesis of Copper Oxide nanoparticles from Syzygium alternifolium (Wt) Walp: Characterization and evaluation of its synergistic antimicrobial and anticancer activity. Appl Nanosci 7: 417-27.

17. Umera BAK, Senthil R (2015) Phytochemical Evaluation of Different solvent Extracts of a Traditional medicinal plant. World J Pharm Res 4: 793-801.

18. Jaiswal BS (2012) Solanum Torvum: A Review of its traditional uses, phytochemistry and pharmacology. Int J PharmBio Sci 3: 104-11.

19. Ndebia EJ, Kamga R, Nchunga-ANB, Analgesic and anti-Inflammatory properties of aqueous extract from leaves of Solanum Torvum (Solanaceae). Afr J Tradit Complement Altern Med 4: 240-4.

20. Rama S, Sourabh D, Sharma BK (2012) Antimalarial plants of northeast India: An Overview. J Ayuv Integr Med 3: 10-15.

21. Bhadra MP, Sreedhar B, Patra CR (2014) Potential theranostinostics application of bio-synthesized silver nanoparticles (4-in-1 system). Theranostics 4: 316-35.

22. Pulicheria Y, Thirumalandhuni V, Yagani JR, Palempalli UMD, Golla N, et al. (2018) Cost Effective, Green Synthesis of Copper Oxide Nanoparticles Using Fruit Extract of Syzygium alterniflium (Wt.) Walp., Characterization and Evaluation of Antiviral Activity. J Cluster Sci 28: 743-55.

23. Ezealisiji KM, Noundou XS, Ukwueze SE (2017) Green synthesis and characterization of monodispersed silver nanoparticles using root bark aqueous extract of Annona muricata Linn and their antimicrobial activity, Appl Nano Sci 7: 905-11.

24. Mohammed NT, Saba TT (2017) The Effect of Copper Nanoparticles on Liver Function and Some Hematological Parameters in Rat. Int J Sci Res 6: 2265-8.