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Green Synthesis of Copper Oxide Nanoparticles from Magnolia Champaca Floral Extract and its Antioxidant & Toxicity Assay using Danio Rerio

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Abstract: ***The biosynthesis of copper oxide (GS-CuO) nanoparticles utilizing Magnolia champaca floral extract was studied, where the Magnolia champaca was used for the reduction of precurosor to elemental CuO nanopartciles which also provides stabilization. Physiochemical properties of GS-CuO nanoparticles were described utilizing analytical strategies like UV-Vis, XRD, FT-IR, SEM, TEM, Zeta potential and DLS analysis. The UV-Visible spectrum gave maximum absorbance in the scale of 250-350 nm. The biosynthesized GS-CuO was crystallite in nature and it was investigated by XRD and was verified with JCPDS NO: 89-589. FT-IR analysis spectrum at 3302 cm^{-1} is assigned for alcoholic hydroxide group, 1022 cm^{-1} correspondings to CH_3 shaking vibration respectively. The morphology of biosynthesized nanoparticles was between 20 to 40 nm and spherical shape was investigated utilizing TEM. The antoxidant potentiality of GS-CuO was evaluated by DPPH, ABST test, that demonstrated inhibition values at 76.30% and 66.46% respectively. Toxicity quality examination was performed utilizing morphological investigation, incubating, and viability rate examination on zebrafish embryonic model. The toxicity quality assessment with zebrafish uncovered organ advancement with various viability and hatching speed at 48 and 72 hpf with LC50 of 500 ± 15 mg/L.***

Keywords: GS-CuO nanoparticles; Antioxidant activity; zebrafish embryos; Toxicity analysis

I. INTRODUCTION

In the present situation, metal oxide (CuO) nanoparticles have increased huge intrigue and consideration for the reason of their physico-chemical property [1,2]. Amalgamation of CuO nanoparticles has been considered to be better than other metal oxide nanoparticles due to their tremendous potential applications in the previous decade [3]. Nature has created diverse methods to synthesize the nanoscale compounds which are added to improve the research development in the biosynthesis of nanomaterials [4]. Typical physical and chemical have been significantly replaced with biological synthesis methods synthesis using microorganisms, enzymes, and plant extract as an eco-friendly alternative approach [5,6].

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Using plants for biosynthesis of nanoparticles is a particularly useful approach than other biological ways as it eliminates the challenges associated with microbial culture maintenance [7]. Nanomaterials exhibit enhanced properties which could be of great interest to us instead of their bulk counterparts [8]. Synthesis of nanoparticles in a controlled shape and size
manner allows manipulation at the atomic scale. CuONPs has magnificently emerged as one of the most researched metal oxide nanoparticles due to their captivating cytotoxic, antimicrobial, optical, electronic properties and thus, they are used in semiconductors and intrauterine contraceptive devices [9]. *Magnolia champaca* is a tropical plant and has been integrated with traditional healthcare system all over the world. Different parts of *Magnolia champaca* is known to be used as ayurvedic medicine in India for curing specific disease ailments[10].
The floral extract of *Magnolia champaca* has antiulcer, antidiabetic and anti-inflammatory properties and hence used for the treatment of ulcer, skin disease and wounds [11,12] . Rajshree Sinha et al. performed scavenging activity of reduced power assay on a floral extract of *Magnolia champaca* is a higher percentage of inhibition 90.20% in comparison with ascorbic acid [13,14]. Bangladeshi medicinal plants which are generally utilized in various sicknesses are also assessed for antioxidant activity via DPPH and ABST analysis [15].
Researchers believe the zebrafish is an ideal model for in vivo advancement of new drugs. Most researchers use zebrafish embryos simply because of their rapid growth, transparency and their small size [16]. Their fast-growing tissues provide an opportunity to predict the reaction properties of new drugs [17]. Their resulting information depends on the size and concentration of NPs, indicating the effect of NPs on the function of the nucleus [18].
Various size of metal containing NPs like Cu, Se, Zr, CdS and Ag with various shape and size of macro forms of the metals [19]. the set of metal composite NPs used as a model in the research of acute and sublethal toxicity in zebrafish embryos [20].
The (Danio rerio) Zebra fish has risen as critical form for drug delivery and toxicological screening in preclinical investigations. The model is adaptable for physiological, biological and molecular alteration. The Organization for Economic Co-activity and Development (OECD) gives a standard rule to assess the embryo toxic impacts of compounds in 96 h of fetus formative stages.

The zebrafish utilized are not just restricted to toxicity screening, but researchers are on the other hand building up the transgenic zebrafish model through genetic adjustment and focuses on transformation for diseases, for example, digestive system, cardiovascular, neurological, diabetes, malignant growth and inflammation [21]. In this current study, we are orchestrated Copper Oxide nanoparticles utilizing floral part of *Magnolia champaca.* Synthesized CuO NPs was characterized by XRD, UV, FTIR, TEM, SEM, and DLS studies. Furthermore, the biological efficacy of CuONPs was examined with anti-oxidant activity. Toxicity analysis was studied using zebrafish embryo as a model animal.

II. EXPERIMENTAL SECTION

A. Preparation of Magnolia champaca extract

The *Magnolia champaca* flowers were gathered from VIT garden and it was washed thrice with dis.H_2O and dried at room temperature. Then, 1:10 ratio of dry flower powder and distilled water was maintained, followed by boiling for 30 min. After 30 min, the solution turned into a yellow colour called *Magnolia champaca aqueous extracts.*

B. Synthesis of CuO nanoparticles

3 mM concentration of copper acetate aqueous solution was added into 10 ml aqueous extracts. Furthermore, these mixtures were kept at magnetic stirrer (37°C) for 24 h. (Fig. 1) After 24 h the solution was turned into brown colour was confirmed the biosynthesis of CuO nanoparticles. The reaction mixture compound was centrifuged at 3000 rpm for 5 min. Finally, the synthesized CuO nanoparticles were dried overnight in a hot air oven at 60°C.

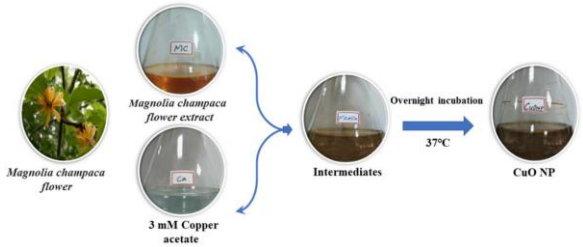

Fig. 1 Schematic diagram of the green synthesis of CuO nanoparticles from the floral extract of Magnolia champaca

C. Physicochemical characterization of CuO NP

The Magnolia champaca synthesised CuO nanoparticles were characterized by UV-Visible spectrophotometer (Cary 5000, Agilent, USA). The cubic crystalline size of the nanoparticles was identified by XRD (XRDBRUKERD8 ADVANCE). FTIR spectroscopy was examined in the range of 400-4000 cm^{-1} to identify the functional group which was presented into the flower extracts. The size of particles was analyzed using SEM (Zeiss EVO18), the presence of Cu and O_2 elemental analyzed by EDS. The stability of the Copper oxide nanoparticles was determined using zeta potential and the particle size of the nanoparticles analyzed by DLS (HORIBA SZ100).

III. IN VITRO ANTIOXIDANT ACTIVITY

A. DPPH activity

The antioxidant potentiality of *Magnolia champaca* extracts mediated CuO nanoparticles was assessed by DPPH technique [22]. Various concentrations (100, 200, 300, 400 and 500 µg/mL) of CuO nanoparticles were reacted with a constant range of (500 µl) DPPH solution. Finally, the mixture was made with 3 ml of methanol. For positive control ascorbic acid was prepared and used same concentration. This entire solution mixture was well shaked and maintained for 30 min in the dark condition at normal room temperature. After 30 minutes incubation, the absorbance was measured at 517 nm using UV-Visible spectrophotometer.

Radical scavenging activity (%) = ((control-test)/control) ×100 -------1

B. ABTS radical scavenging assay

The in-vitro antioxidant action of ABTS was formed. 2 mM ABTS solution with 17 mM, (0.3ml) potassium persulfate was kept in the dark condition for 12-16 h at 28°C. This solution was diluted in ethanol at 30°C. Then, the ABTS solution was added to various concentrations of 100- 500 µg/ml CuO nanoparticles and kept in the dark conditions at normal room temperature for interval of 30 min. For positive control ascorbic acid was prepared and used same concentration. After 30 min, the solution was measured at 734 nm using UV-Visible spectrophotometer.

ABTS (%) = ((control-test)/control) ×100 ------2

IV. ZEBRAFISH AND EMBRYO MAINTENANCE

All the experiments was accomplished by appropriate animal practice rules and policy of OECD. Zebrafish were acquired from a native aquarium fish seller, the zebrafish wild-type AB strain was maintained. Breeding of fishes was encouraged by maintaining females and males in a 3:1 ratio. Then photoperiodism was continued by maintaining them for 10 hours in darkness and 14 hours in light. Before breeding the water, temperature was kept at 26 ± 2°C supplemented with a proper foodstuff along with *Artemia*. Eggs are collected in early morning and cleanse repeatedly and it was further reared up in E3 medium (5 mmol/ L Sodium chloride, 0.18 mmol/L Potassium chloride, 0.33 mmol/L Calcium chloride, 0.33 mmol/L Magnesium sulfate) containing 2 µg/mL gentamycin. The embryos originated from natural spawning and it was incubated at 28.5°C.

V. TOXICITY STUDY OF GS-CUO NPS IN EMBRYO ZEBRAFISH AND LARVAE MODEL

The Copper Oxide nanoparticles were tried by in-vivo toxic quality examinations in zebrafish hatchlings and developing life model. In brief, 30 zebrafish embryos of 24-hours post-fertilization (hpf) were open to Green Synthesizes Copper Oxide nanoparticles at an array of concentration of 450 mg/L to 500 mg/L in E3 medium for period of 72 hours.

A photoperiod of 10 hours in darkness and 14 hours in light and the setup was incubated at 28 ± 1°C. Observation by microscopy was done at every intermission to envision the growing and morphological variations. The hatching assess was resolute as several hatched embryos by 72 hpf as equated to the unprocessed group. The death rate was stated and several expired embryos after 72 hpf as compared to the control group. All the experimental tests are performed in triplicates.

VI. RESULTS

A. UV-Visible analysis of CuO Nanoparticles

The solution mixture turns brown colour reaction as the certain time (8 h) which indicates the development of biosynthesized Copper oxide nanoparticles. The CuO nanoparticles were determined by UV-Vis spectrophotometer at a scanning range from 200 nm– 600 nm. The broad peak shown at the range of 250-350 nm confirmed the CuO nanoparticles were synthesized using *Magnolia champaca* shown in (Fig. 2) Similar results were observed that the range of peak at 285 nm [23].

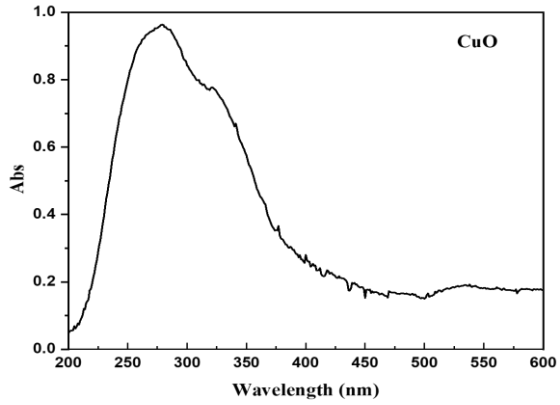

Fig. 2 (A) UV-Vis spectrum of *Magnolia champaca* mediated GS-CuO nanoparticles. (B) Zeta potential of *Magnolia champaca* mediated GS-CuO nanoparticles determined by Dynamic light scattering

B. XRD analysis of Magnolia champaca mediated CuO NP

The cubic crystalline structure of CuO nanoparticles was examined using X-ray Diffraction technique. As demonstrated in (Fig. 3) the characteristic X-ray Diffraction peak were detected at 32.05, 35.24, 37.16, 48.83, 53.02, 58.89, 61.30, 65.12, 67.19, 72.54 and 75.33 correspondings to (110), (111), (200), (202), (020), (202), (113), (022), (113), (311) and (004) planes, respectively. The standard size of the particles of GS-CuO nanoparticles was considered by using the Debye-Scherrer equation

$$D = \frac{k\lambda}{\beta cos\theta}$$

D = average Crystallite size k= shape factor (0.94), λ = X-Ray wavelength (λ= 1.5418 Å), β= Line broadening of full width at half maximum (FWHM) in radians, θ = Bragg angle. The Crystallite average particles size of CuO was established to be 35 ± 6 nm.

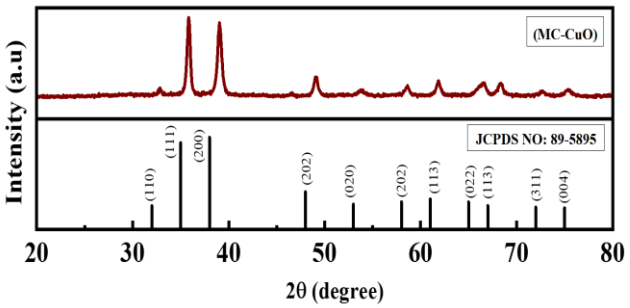

Fig. 3 XRD pattern of *Magnolia champaca* mediated CuO nanoparticles

C. FTIR analysis of Magnolia champaca mediated GS-CuO nanoparticles

The FT-IR measurements were carried out by *Magnolia champaca* floral extracts mediated copper oxide nanoparticles. The FTIR Fig. 4A showed the plant extract having band at 3334 cm^{-1}, 2920 cm^{-1}, 2835 cm^{-1}, 1710 cm^{-1}, 1647 cm^{-1}, 1375 cm^{-1}, 1255 cm^{-1}, 1020 cm^{-1}, and 538 cm^{-1}. The broad peak observed at 1647.21 cm^{-1}corresponds to the C=C stretching vibrations conjugated with C=O. The small peak at 2920 and 1375 resembles to the C-H deformation vibrations of the compounds. The pointed peak at 1020 cm^{-1} indicates the existence of CH_3 rocking vibration. FTIR (Fig. 4B) showed the GS-CuO NP having band at 3302.13 cm^{-1}, 2943 cm^{-1}, 2831 cm^{-1}, 1417 cm^{-1}, 1114 cm^{-1}, 1022 cm^{-1}, 621 cm^{-1}. The peak observed at 2831, and 3302 cm^{-1} corresponds to the -OH stretching vibration when a hydrogen bond is present. The small peaks at 2943 cm^{-1}correspondings to CH_2 extending vibration. Broad peak was observed at 1022 cm^{-1} correspondings to CH_3 rocking vibration. The below 700 cm^{-1} frequencies peak denotes in the infrared spectrum is for Cu-O vibrations.

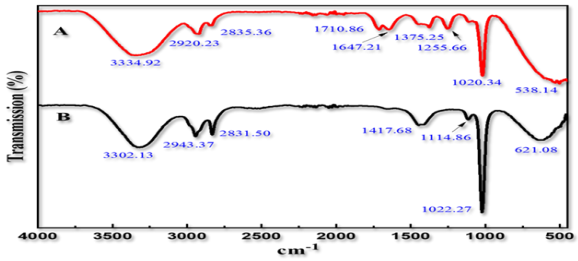

Fig. 4 Physiochemical characterization of GS-CuO nanoparticles. (A) plant extract of FTIR spectrum at 500 cm-1 to 4000 cm-1. (B) Copper oxide nanoparticles

D. Size and shape determination of CuO nanoparticles

The morphological examination of biosynthesized CuO nanoparticles was done with SEM images shown in (Fig. 5A). The CuO nanoparticles were established to be a spherical shape and agglomerated. Similar reported was observed into the *Magnolia champaca* plant extracts mediated copper oxide nanoparticles [24]. TEM images are shown in (Fig. 5B) also exhibited synthesized Copper oxide nanoparticles were spherical having a size assortment of 20 nm-40 nm of GS-CuO nanoparticles. EDAX analysis was studied and exhibited by Cu and O element was presented in CuO nanoparticles shown in (Fig. 5C). In this figure, we are examined and reported to the synthesized nanoparticles were pure and without any other impurities.

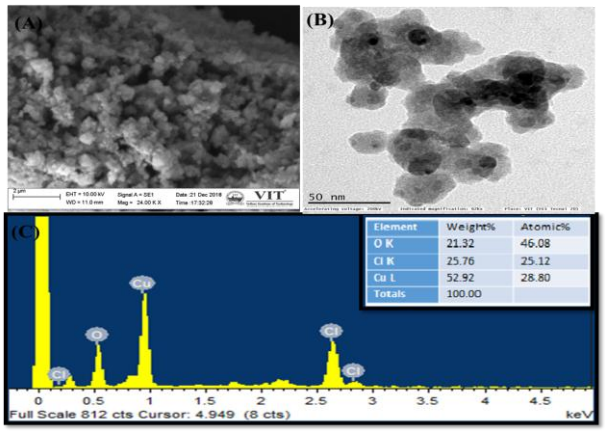

Fig. 5 Size and shape determination of GS-CuO nanoparticles. (A) SEM (Scale bar of 1µm) (B) TEM 50nm. (C) EDS analysis of GS-CuO nanoparticles

E. Particle stability and size determination of GS-CuO nanoparticles by Dynamic light scattering

The biosynthesized nanoparticles stability was studied by Zeta potential. Hydrodynamic diameter determined by DLS using the freshly prepared GS-CuO nanoparticles was established to be 85.7±20 nm, as shown in (Fig. 6A). This size value almost got an agreement with the TEM size of *Magnolia champaca* synthesized GS-CuO. *Magnolia champaca* synthesized GS-CuO were shown the zeta potential value was -18.0 mV shown in (Fig. 6B).

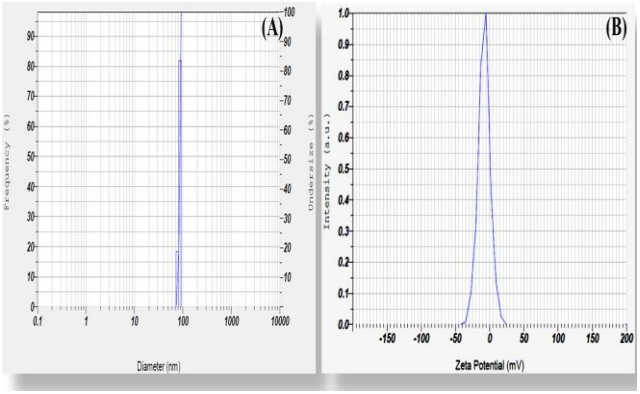

Fig. 6 Particle size (A) determination of GS-CuO nanoparticles and (B) Dynamic light scattering

F. In vitro antioxidant activity

The antioxidant activity of the GS-CuO nanoparticles investigated against DPPH free radicals was revealed in (Fig. 7A). Maximum radical inhibition (76.30%) was observed at high concentration (500 µg/mL) of with *Magnolia champaca* synthesized GS-CuO. However, when compared to the % of DPPH activity of standard antibiotic ascorbic acid showed a slightly lesser activity (66.41%) at 500 µg/mL concentration. However, ascorbic acid used as standard showed a somewhat lower inhibition effect (66.46%) at 500 µg/mL concentration. The evaluated through ABTS assay has reported maximum inhibition rate up to 88.53% (Fig. 7B).

On the basis of the IR result, it could be confirmed that polysaccharides and phenolic acids decorated on the GS-CuO have an essential role in improving the antioxidant effect. DPPH and ABTS techniques were used expansively a free-radical steady to assess decreasing materials and it was beneficial solution for examining free-radical scavenging action of the copper oxide nanoparticles. ABTS activity includes a radical, which is chemically shaped, removing the color in its non-radical system and is frequently utilized for selecting from complicated compound such as GS-CuO.

Dilaveez [25] studied *Moringa oleifera, Hibiscus rosa-sinensis, Murraya koenigii, Azadirachta indica, and Tamarindus indica* aqueous extracts facilitated copper oxide nanoparticles and its antioxidant potentiality. They reported phenolic compounds presented in nanoparticles are mainly can play a significant role in antioxidant activity. Rajshree Sinha et al., performed scavenging activity of reduced power assay on a floral extract of *Magnolia champaca* is a higher percentage of inhibition of 90.20% in comparison with ascorbic acid. Indian medicinal plants which are by tradition used in different sicknesses are evaluated for radical scavenging activity of DPPH, ABTS activity [26]. Moreover, the green synthesized nanoparticles show potential antioxidant activity due to their functional groups presented into the nanoparticles.

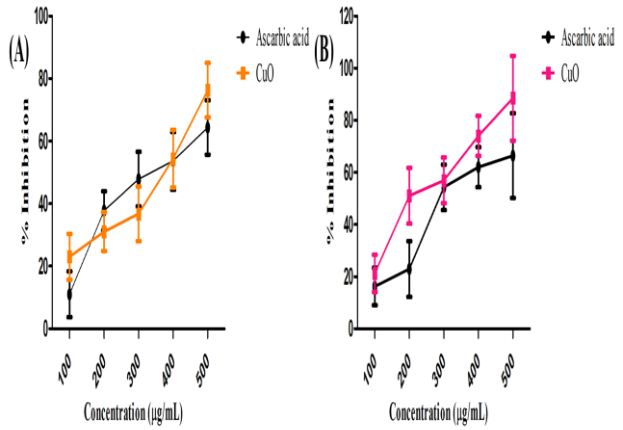

Fig. 7 Effect of copper oxide nanoparticles on in vitro antioxidant activity using DPPH (A), ABTS (B) assays

G. Toxicity of embryonic zebrafish from Magnolia champaca mediated CuO NP

Evaluation of toxicity GS-CuO nanoparticles was prepared with embryonic zebrafish in vivo model, due to advantages like their short life cycle, cost impact and hereditary comparability with human [27]. Zebrafish have been applied for nanotoxicology studies, in our research intense toxic quality analysis was performed in Magnolia champaca mediated GS-CuO nanoparticles and observed for their differences in contrast with the untreated embryonic organisms assumed as control for the investigation. In Fig. 8. The embryos in untreated or treated conditions resulted to be at 450 mg/L, and 550 mg/L concentrations when exposed to GS-CuO nanoparticles. Similar results of analyses for viability rate were resolved in a group of 30 appeared as shown in (Fig. 9A). As well as associated between zebrafish embryonic LC50 estimations of 500 mg/L with the hatching assess were additionally determined as the embryos hatched in the level of entire uncovered embryos appeared in (Fig. 9B). The nanoparticles were collected at the skin surface, and chorion of 24, 48 and 72 hpf and embryos are treated separately.

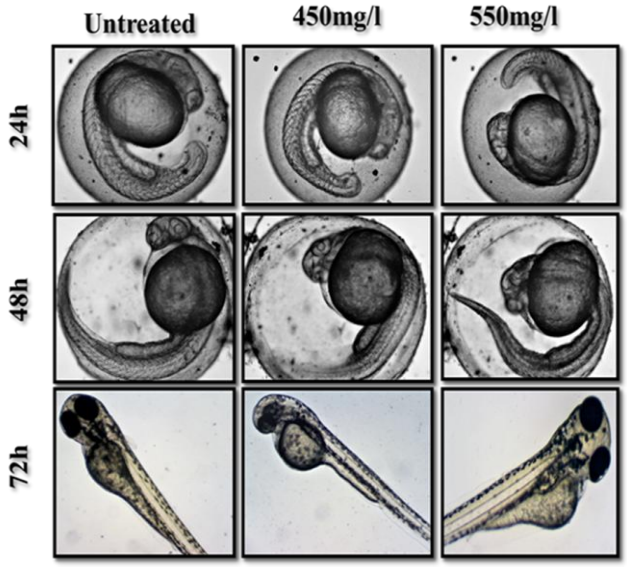

Fig. 8 The morphological analysis of Zebrafish embryos exposed to different the concentration of *Magnolia champaca* mediated GS-CuO nanoparticles

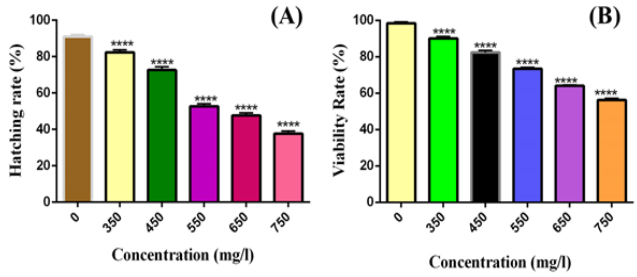

Fig. 9 (A) Viability rate. (B) Hatching percentage of Zebrafish embryos exposed *Magnolia champaca* mediated GS-CuO nanoparticles at different hours of post-fertilization (hpf).

The values were presented as the mean ± SD of three independent experiments. *P<0.05 denotes the significant change from untreated embryos respectively as obtained from ANOVA analysis. Several *presents the degree of significance

VI. DISCUSSION

In this study, Copper Oxide nanoparticles has been prepared using therapeutic plant extract and their toxicity on embryonic zebrafish. The floral extract from *Magnolia champaca* has been well documented for its medicinal assets worldwide. The floral extract changed its colour from yellow to black and the precipitation obtain at the last stage of the incubation period had confirmed the biosynthesis of GS-Copper Oxide nanoparticles.

The literature has also reported the occurrence of diverse biomolecules like starch [28], flavanol glycosides and phenol. They are biomolecules that attribute to the stabilization and reduction of CuO nanoparticles for copper acetate salt. Different techniques characterized the mediated GS-CuO nanoparticles for its morphology, and it was found that they fall in the series of 20-40 nm as resolute by TEM and SEM images (Fig. 5). The existence of Cu and O_2 proved the development of particles identified by EDS analysis. The SPR peak at 320nm, as shown in (Fig. 2) demonstrated that the nucleation of the nanoparticles was determined by its stability taken at an interval of time of 8hrs. The function of zeta potential is to discover the electrophoretic mobility of particles in the medium [29]. Our result was established to be -08 ± 06 mV as shown in Fig. 5B for GS-CuO nanoparticles.

DPPH and ABTS were used widely as a steady free-radical to assess falling material and it are beneficial reagent for examining free-radical scavenging action of the copper oxide nanoparticles. ABTS has an excellent antioxidant property [15]. The ABTS scavenged of percentage effectiveness by copper oxide nanoparticles were established to rise with rising concentration. Toxicity of nanoparticles with current changes in development, morphological and physiological level was assessed for GS-CuO nanoparticles in embryonic zebra fish at various concentrations. The structural change was seen at 24, 48 and 72 HPF of embryonic zebra fish on the revelation of GS-CuO nanoparticles at a lower and higher concentration. As shown in (Fig. 8) the low-level concentration at 450 mg/L, the yolk sac was found to be in abnormal from, and at high-level at 550 mg/L, the pericardial edema was found to be in an abnormal condition [30,31]. The hatching enzyme molecules like medaka high choriolytic enzyme (MHCE) is assumed to be interacting with the GS-CuO nanoparticles, this eventually lowers the hatching rate of the embryos, thereby reducing the mortality rate and increasing the hatching of the embryos. GS-CuO nanoparticles showed LC_{50} of 500 ± 15 mg/L, which were fairly high as paralleled to the LC_{50} of available Copper Oxide nanoparticles [30].

VII. CONCLUSION

In this present study, a green, environmentally benign approach for GS-CuO nanoparticles synthesized by *Magnolia champaca* floral extract. The physiochemical characterization investigation was confirmed the synthesized GS-CuO nanoparticles were nanosized. DPPH and ABTS assay were studied and observed the synthesized nanoparticles best choice of anti-oxidant activity. Antioxidant molecules which are depicted in FTIR were responsible for the various biological activities. Synthesis of GS-CuO nanoparticles can be a possible suspension with state delivered and it can be used in absolute concentration. Toxicity assessment of *Magnolia champaca* mediated nanoparticles was exhibited the nontoxic material. These conclusions will cover the pathway for advance research in CuO nanoparticles toxicity to Zebrafish.

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