Evaluation of copper oxide nanoparticles as antimicrobial fillers for nanofilled composite resin

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ABSTRACT: The aim of this research paper is toevaluate the influence of adding different concentrations of copper oxide nanoparticles to nano-filled composite regarding its antibacterial activity and physical properties. Copper nanoparticles were fabricated by using hydrothermal chemical precipitation technique, then characterized by; Transmission electron microscopy, X-ray diffraction, Ultra-Violet/visible analysis, and Fourier transforming infrared spectroscopy. Z350xt composite was modified with two different concentrations of copper oxide nanoparticles; CuO 0.005% wt. and CuO 0.01% wt, then the modified composite was assessed for degree of conversion, mechanical properties, color change and antibacterial activity against both E.coli and S.mutans.

KEYWORDS: Copper oxide, Antibacterial activity, Modified composite, E.coli, S.mutans.

I- INTRODUCTION

Resin composites over decades were demanded for its esthetic property. As it is not only the first option for restoring the anterior teeth, but also its use in posterior teeth restoration is increasing. Over the past decades the scientist's main goal was improving monomer, fillers, or the initiating system. First, the size of fillers decreased continuously to overcome the problem of shrinkage, until they reached the nano-sized fillers, and it was also introduced in hybrid state. Second, the monomers were improved as they are the key constituent to provide the excellent bond with resin composite. It was reported that decrease in monomer viscosity increased the mechanical properties of dental composite resin especially, degree of conversion. Third, the initiating system was improved to increase wear resistance, degree of conversion, and reduce residual monomers from polymerization process. And finally, the competition now in the field of research is to produce antibacterial composites in order to overcome the future failure due to secondary caries produced by bacterial accumulation around the restoration [1-5].

Nanoparticles are developed with unique properties that make them desirable in materials science and biology [6]. Among various nanoparticles, copper oxide was used in this study for its known antibacterial property. Copper oxide nanoparticles has attracted particular attention because it is the simplest member of the family of copper compounds and shows a range of useful physical properties and antimicrobial properties as; it can prevent microorganisms from attaching, colonizing, spreading, and forming biofilms[7-9].

II- MATERIALS AND METHODS
Table (1): Materials used in the study

Material	Manufacturer	Batch No.
Z350xt composite	**3M Filtek, USA**	**775639**
Copper acetate monohydrate (Cu (CH$_3$COO)$_2$·H$_2$O),	**Merck, Germany**	**102710**

Sodium hydroxide (NaOH)	Merck, Germany	106498

1. Copper oxide nanoparticles (CuO NPs) preparation

CuO NPs were prepared using hydrothermal chemical precipitation technique.An aqueous solution of (Cu $(CH_3COO)_2 \cdot H_2O$) was prepared with a volume of 100 mL and a concentration of 0.1 M. The aforementioned solution was thereafter agitated with strong intensity at a rotational speed of 900 revolutions per minute (rpm) with a DLAB (MS7-H550-S) magnetic stirrer. Subsequently, an aqueous solution of NaOH was incrementally introduced until the pH reached a value of 12. The molar ratio between NaOH and Cu $(CH3COO)_2 \cdot H_2O$ was determined to be 1:2. After 4 hours of stirring at a temperature of 60°C, the color of the fluid underwent a transition from blue to black. This change was accompanied by the creation of a precipitate, which suggests the production of nano-particles. Throughout the synthesis process, a consistent temperature and stirring rate were preserved. Collection was achieved through centrifugation for 20 minutes at a speed of 10,100 revolutions per minute, which was repeated three times. Then, collected CuO NPs were washed with distilled water, the dried for 4 h at a temperature 60°C [10].

2. Physio-chemical characterization of the synthesized nano-particles

The produced CuO NPs were characterized by UV-Visible absorption spectroscopy using a spectrophotometer (Bio Aquarius CE 7250, United Kingdom) throughout a wavelength range of 200-700 nm to ensure chemical reduction [11]. The appearance and size of the produced NPs were analyzed using transmission electron microscope (Zeiss-EM10C-100 KV electron microscope, Germany). The specimens were deposited into TEM copper grids that had been coated with carbon using the drop-casting method, as they are thin and highly transparent to electrons, offering fine grain and low contrast that doesn't interfere with specimens' spectrum [12]. Afterward, the prepared specimens were allowed to air dry to prepare NPs for imaging. Fourier transform infrared (FT-IR) spectroscopy, utilizing the Nicolet (Thermo-Fisher-USA), it was employed to analyze and identify the existence of particular chemical bonds (functional groups) located on the surface of CuO NPs throughout the spectral range from 400 to 4000 cm^{-1}[13].

3. Modification of composite by CuO NPs

CuO NPs were added into the composite resin Filtek Z350 based on the inclusion of weight percentage of particles. CuO NPs were weighed by 0.005% and 0.01% (wt%). The NPs were incorporated into the composite resin by manual mixture for 40 seconds, using a metal spatula and a glass plate. After modification of composite, three samples were prepared (one for each group) in a plastic disc mold with dimension (4mm diameter x 2mm thickness) and light cured from each side for 20 second [14].

4. Grouping of modified composite:

The Nano filled composite (Filtek Z350) was employed as a control group and modified composite with CuO NPs with concertation 0.005% and 0.01% (wt%) for experimental groups. A total of 3 groups were tested in this study as follows:**Group 1:** Nano-filled composite (Filtek Z350) as a control group,**Group 2:** Composite modified by CuO 0.005 and **Group 3:** Composite modified by CuO 0.01.

5. Sample preparation

A total number of 150 specimens (n=150), disc shaped samples were prepared in the study (50 for each group). Ten specimens for each group were used in each test by using a split plastic mold. All procedures were carried out by the same operator using standardized methods. First, a transparent mylar strip was applied against a microscopic glass slide, then the mold was fixed on them. The powder of nanoparticles and nano-filled composite were mixed for 40 second on a mixing pad. The mixed material was transferred to the molds with a plastic instrument from one side of the mold till the material extruded from the other side. The completely filled mold was covered by another mylar strip and a glass slide. The slide was pressed lightly and constantly by hand to allow excess material to be extruded, resulting in a smooth surface. After removing the glass slide, the samples were cured for 30s by the light-guide tip of the LED light cure with wavelength 400-500nm, then the bottom slap was removed and the curing repeated from this side. The light intensity of the lead light cure was regularly checked by radiometer (SDI LED Radiometer). The prepared samples were stored in distilled water for 24 h at 37°C to complete the polymerization [15].

6. Characterization of modified nano-filled composite

a. Degree of conversion

A total number of 30 disc-shaped specimens (10 specimens for each group) were prepared in a plastic split disc mold (5mm diameter and 2mm thickness). After 24 hours storage, each specimen was manually pulverized into fine powder by using a mortar and pestle. Fifty micrograms of the ground powder were mixed with potassium bromide (KBr) in an amount that is ten times as the specimen powder. Both powders were then compressed in to disc form. The compressed disk was irradiated by the infrared spectrum of FTIR spectroscopy. The absorbance peaks of infrared rays causing stretching vibration of the aliphatic C=C double bond at 1632 to 1639 cm^{-1}, aromatic C….C double bond (which is used as internal reference of nano-filled composite) at 1609 to 1620 cm^{-1}[16].The percentage of DC for modified nano-filled composite specimens was calculated by using the following equation:

$$DC = \left(1 - \frac{R(cured)}{R(uncured)}\right) \times 100$$

b. Diametral tensile strength testing

A total number of 30 disc-shaped specimens (10 specimens for each group) were prepared in a plastic split disc mold (6mm diameter and 3mm thickness). Diametral tensile strength was examined and data were collected using computer software called Bluehill Lite from Instron®. Each sample was mounted separately on computer-controlled materials testing equipment (Model 3345; Instron Industrial Products, Norwood, MA, USA) with a loadcell of 5 kN. Then, using a stainless-steel rod with a flat end that was 10 cm wide by 5 cm long and coupled to the machine's upper movable compartment, the samples were statically loaded diametrically until they failed [17].

The maximum failure load was recorded in N. The diametral compressive strength was calculated from the following equation:

$$\delta = 2P/ \pi D T$$

Where ; δ = Diametral compressive strength (MPa)

P = load at failure (N)

π = 3.14.

D = disc diameter(mm)

T = disc thickness(mm)

c. Compressive strength testing

A total number of 30 disc-shaped specimens (10 specimens for each group) were prepared in a plastic split cylinder mold (4mm diameter and 6mm thickness). Compressive strength was examined and data were recorded using computer software (Bluehill Lite; Instron Instruments). Each sample was mounted separately and vertically on a computer-controlled material testing machine (Model 3345; Instron Instruments Ltd., USA) with a load cell of 5 kN. Following that, the samples were statically loaded (in a compression manner) using stainless steel rods ending with flat plates (40 mm x 60 mm) attached to the upper moveable compartment of the machine, and this process was continued until failure. The maximum failure load was calculated in N. The compressive strength was calculated from the recorded peak load divided by the sample surface according to the following equation;

Compressive strength (CS) = 4P/πd^2

Where P is the load (N) at the fracture point and d is the diameter (mm) of the cylindrical specimen [18].

d. Evaluation of color change

A total number of 30 disc-shaped specimens (10 specimens for each group) were prepared in a plastic split mold (4mm diameter and 2mm thickness). A Spectrophotometer was used to gauge each specimen's color. The International Commission on Illumination (CIE) standards for color measurement were followed. A reflective spectrophotometer (X-Rite, model RM200QC, Neu-Isenburg, Germany) was used to measure the color of the specimens. The specimens were perfectly aligned with the instrument and the aperture size was adjusted at 4 mm. A white background was chosen, and measurements were taken in relation to the CIE standard illuminant D65 using the CIE L*a*b* color space[8,19].

The color changes (ΔE) of the specimens were evaluated using the following formula:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Where: L* = lightness (0-100), a* = (change the color on red/green axis) and b* = (color variation on yellow/blueaxis).

e. Antibacterial testing

Microbial strain and growth media:

The antibacterial activity was measured against Streptococcus mutans (NCTC No. 10449) and E.coli (EMCC No. 1815). Bacterial strains were transferred from stock culture to Brain Heart Infusion (BHI) broth and incubated at 37° C for 24 hours. The bacterial growth was measured by the presence of turbidity in the broth after incubation. Turbidity was evaluated visually by comparing the inoculum tube and the standards against a

contrasting black card. The antibacterial test was then done using Agar disc-diffusion test.

Agar disc-diffusion

A total number of 30 disc-shaped specimens (10 specimens for each group) were prepared in a plastic split disc mold (4mm diameter and 2mm thickness).The specimens underwent sterilization in an autoclave at a temperature of 121 ◦C for 15 minutes. $1-2 \times 10^8$ CFU/ml of bacterial suspension were placed on 150 mm of each solid agar plate. The composite resin discs were placed on the solid agar surface. A group of 3 plates served as a positive control. All plates were maintained in room temperature for 2h in order to permit diffusion of the test materials, and then incubated at 37 ◦C for 24h in an incubator. The diameter of the halo around the specimens (inhibition zone) was measured in each plate by same operator to confirm the standardization and accuracy by millimeter ruler [20,21].

III.RESULTS

1. Physicochemical characterization of CuO nanoparticles

a. Transmission electron microscopy (TEM)

TEM analysis of CuO NPs is shown in **(Figure 9).** CuO NPs morphology is shown in **(Fig. 9A),**CuO NPs are nearly rectangular shape randomly distributed with a dimension ranging from 60 to 170 nm as shown in **(Figure 9B).** SAED pattern in **(Figure 9C)** confirms the crystallinity of CuO NPs.

b. X-ray diffraction (XRD) analysis

The XRD spectra of CuO NPs are shown in **(Figure 9D).** The maximum Bragg reflection peaks appeared were at 35.32 ˚, 38.50 ˚, 48.49 ˚ and 61.23 ˚, which are attributed to (002), (200), (202) and (113) crystal planes respectively. The CuO NPs sharp peaks showed its crystallinity. The absence of any additional peaks demonstrated the purity of the samples.

c. Fourier transforming infrared analysis (FTIR)

All the FTIR spectra of the produced CuO NPs are shown in **(Figure 9E).** The FTIR spectrum shows transmittance peaks at 3540, 1638, 1380, 1026, and 620 cm^{-1}. A peak in transmittance at 3540 cm^{-1} indicates the hydroxyl (O-H) stretching group. A significant stretch of the carbonyl (C=O) group is observed in the band at 1640 cm^{-1}. The peaks at 1380 cm^{-1} and 1034 cm^{-1} indicate the stretching vibrations of the aliphatic amines (the C-N) bonds as well as the rocking vibrations of their methyl groups(C-H). Copper oxide transmittance peaks appear in the 700-500 cm^{-1} spectral region. The copper oxide (Cu-O) bond is shown by a wide band at 620 cm^{-1}, indicating the production of CuO NPs.

d. UV-Vis spectrophotometry

Chemical reduction of CuO NPs was evaluated by UV/vis spectroscopy as shown in **(Figure 9F).** The absorbance peak appeared with the highest value at a range at 242 to 247 nm for CuO NPs.
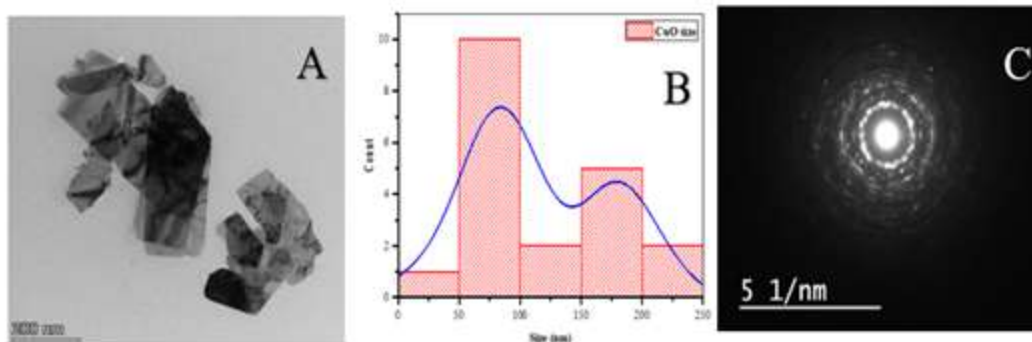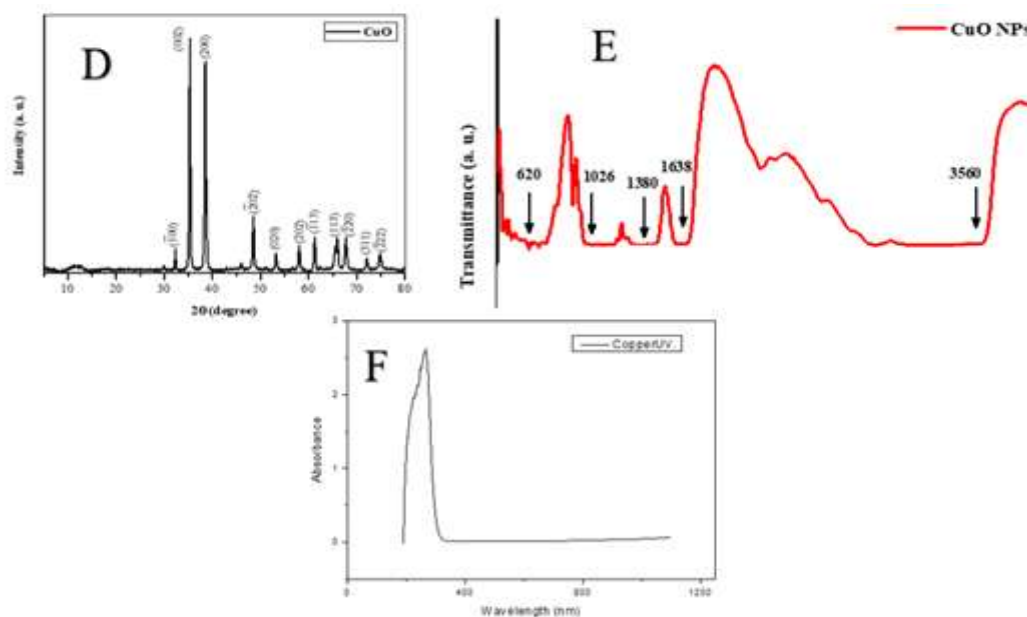
Figure 1: Characterization of produced CuO NPs (A) TEM of CuO NPs, (B) Histogram of CuO NPs, (C) SAED of CuO NPs, (D) XRD pattern, (E) FTIR spectrum, and (F) UV-Vis analysis.

2. Characterization of modified nanofilled composite:

a. Degree of conversion (%)

Means, standard deviation and results of Post Hoc test of DC% of nano-filled composite groups are shown in **Table (2),** and presented graphically in **Figure (2).**One- wayANOVA test showednon-significant difference between tested groups (p=0.154).The highest mean value was recorded in **Group 1** (control) **62.14±1.10%,** followed by **Group 3** (composite modified with Cu 0.01%) **61.63±2.12%,** then **Group 2** (composite modified with Cu 0.005%) **61.29±1.41%.**

b. Tensile strength (MPa)

Means, standard deviation and results of Post Hoc test of tensile strength (**MPa)** of nano-filled composite groups are shown in **Table (2),** and presented graphically in **Figure (2).** One-wayANOVA test showedno significant differencebetween tested groups (p=0.096). The highest mean value was recorded in **Group 1** (control) **45.92± 2.06 MPa**, while in experimental groups, the highest mean value was **Group 3** (composite modified with CuO 0.01%) **42.87±1.98 MPa** then,**Group2** (composite modified with CuO 0.005%) **41.91± 1.94 MPa**.

c. Compressive strength (MPa)

Means, standard deviation and results of Post Hoc test of compressive strength (**MPa)** of nano-filled composite groups are shown in **Table (2),** and presented graphically in **Figure (2).** One-wayANOVA test showedno significant difference between tested groups (p=0.083). The highest mean value was recorded in **Group 1** (control) **192.2±7.49 MPa**, but within the experimental groups the highest mean value was for **Group 3** (composite modified with CuO 0.01%), **183.1±7.17 MPa**, and the least mean value was recorded for **Group 2** (composite modified with CuO 0.005%), **180.7±7.99 MPa**.

d. Color change (ΔE)

Means, standard deviation and results of Post Hoc test of color change of nano-filled composite groups are shown in **Table (2),** and presented graphically in **Figure (2).** One-wayANOVA test showedno significant difference between tested groups (p=0.173). The highest mean value was recorded in **Group 3** (composite modified with CuO 0.01%) **15.99±0.70**, followed by **Group 2** (composite modified with CuO 0.005%) **15.74±0.46**.

Tests / Groups	G1 (control) Mean±SD	G2 (composite modified with CuO 0.005%) Mean±SD	G3 (composite modified with CuO 0.01%) Mean±SD	P-value
Degree of conversion	62.14±1.10[a]	61.29±1.41[a]	61.63±2.12[a]	**0.154**
Tensile strength	45.92±2.06[a]	41.91±1.94[a]	42.87±1.98[a]	**0.096**
Compressive strength	192.2±7.49[a]	180.7±7.99[a]	183.1±7.17[a]	**0.083**
Color change	------------------	15.74±0.46[a]	15.99±0.70[a]	**0.173**

Table 2: Means,SD, results of Post Hoc test and One-way ANOVA of all tests of different groups of composite.

Significance level ≤0.05
Means with same superscript letter are not significantly different.
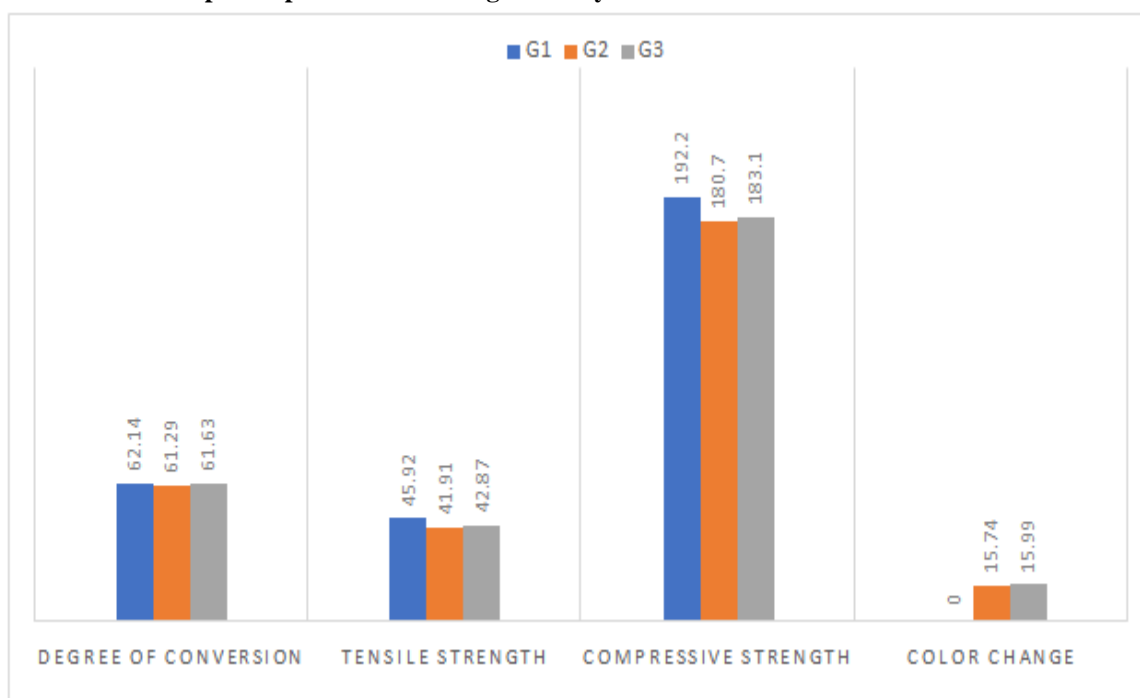

Figure 2: Bar chart comparing mean values of all tests of composite different groups.

e.   Antibacterial activity

The Antibacterial activity of the composite was tested against E. coli (-ve) and S. mutans (+ve) and expressed as a diameter of the inhibition zones in mm. Means, and standard deviation are shown in **Table (3)**, and presented graphically in **Figure (3)** and the inhibition zones were presented in **Figures (4,5,6).** One-way ANOVA test revealed a significant difference between tested groups (p=0.039).

For antibacterial activity against E. coli, modified composite with CuO NPs has no antibacterial activity, no zone was seen in **Group 2** (composite modified with CuO 0.005%)(-ve) and **Group 3** (composite modified with CuO 0.01%)(-ve). While, antibacterial activity against S.mutans**Group 3** (composite modified with CuO 0.01%)(+ve) recorded **8.982±0.19 mm**, followed by **Group 2** (composite modified with CuO 0.005%)(+ve) **7.623±0.21 mm**.

Table 3: Means, SD, results of Post Hoc test and One-way ANOVA of antibacterial activity of different groups of composite.

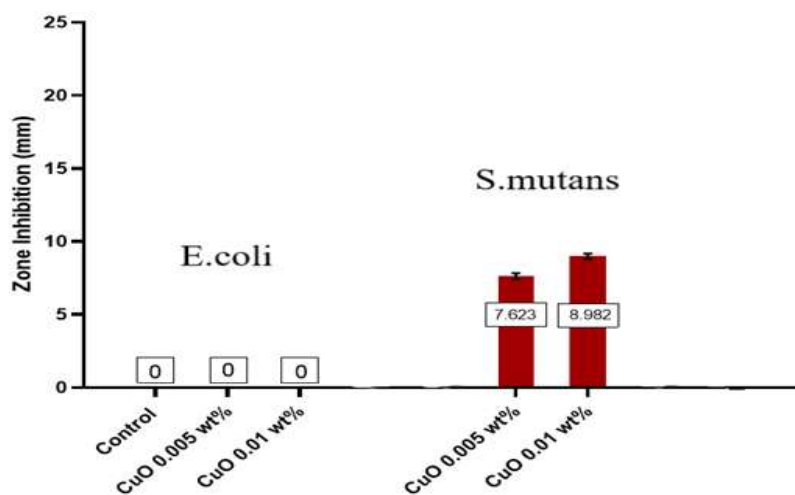| GP/Conc % wt	Strain	Mean±SD	P
G1		0.000±0.00	
G2 G3	E-coli	0.000±0.00 0.000±0.00	0.039[*]
G2 G3	S. mutans	7.623±0.21 8.982±0.19	

Significance level ≤0.05

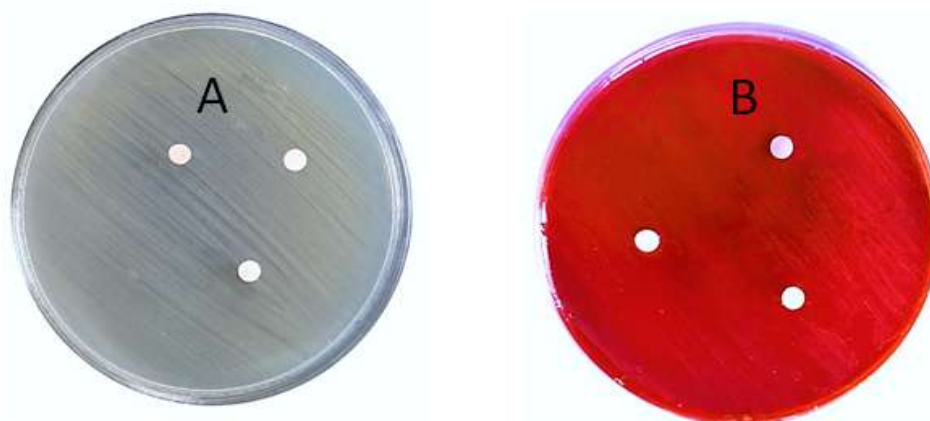**Figure 3: Mean values of antibacterial effect in composite different groups**

Figure 4: Antibacterial activity of Group 1 (control) against A) E. coli, B) S. mutans.
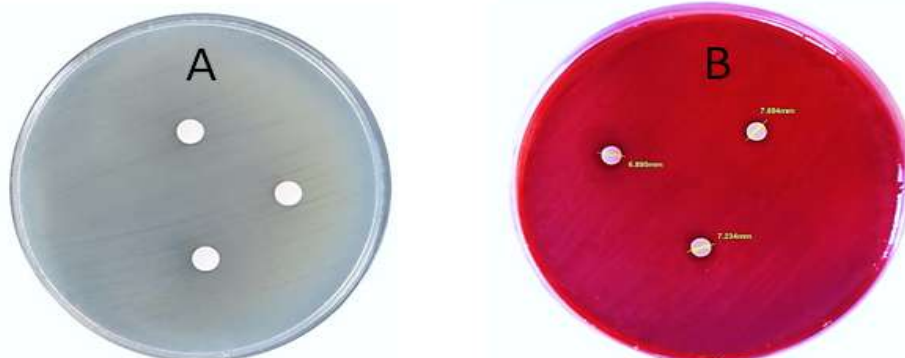
Figure 5: Antibacterial activity of Group2 (composite modified with CuO 0.005%) against A) E. coli, B) S. mutans.

Figure 6: Antibacterial activity of Group3 (composite modified with CuO 0.01%) against A) E. coli, B) S. mutans.

IV. DISCUSSION

The recent scope of interest was fulfilling the perfection for this dental material was improving its antibacterial property So, some of antibacterial agents were used for a long time aiming to reach the optimum results without any change in the esthetical properties of composite. These antibacterial agents were introduced in form of nanoparticles to overcome any surface changes [22].

In this study we reported the antibacterial property of the nano-filled composite resin (Filtek 3M Z350XT) after its modification with different concentrations of CuO NPs, and also evaluated the effect of the nanoparticles on the mechanical and physical properties of resin composite regarding; Degree of conversion (DC%), Tensile strength, Compressive strength, Color changes and Antibacterial activity.

The results of degree of conversion of the present study showed no significant difference between all test groups. As, the highest mean value was recorded for **Group 3** (composite modified with Cu 0.01%) **61.63±2.12%,** then **Group 2 (**composite modified with Cu 0.005%) **61.29±1.41%.** These results confirmed that addition of different concentrations of CuO NPs don't make significant change for the degree of conversion of the restorative material.

A study was reported by **Gutiérrez et al,** [16]**,** added Cu NPs to universal adhesive systems to enhance the anti-bacterial properties of the adhesive system. It was concluded that the addition of Cu NPs didn't change the degree of conversion of the adhesive system, and these results were in accordance with our findings.

Furthermore, a study was conducted by **Gutiérrez et al,** [23]who evaluated ZnO and CuO nanoparticles effect on universal adhesive systems. Their findings are in alignment with our results as they concluded that the addition of both materials

increase the antibacterial activity of adhesive system without affecting its degree of conversion.

The results of tensile strength of the present study showed no significant difference between all test groups. The highest mean value was recorded in **Group1** (control)**45.92± 2.06MPa**, while in experimental groups, the highest mean value was recorded in **Group 3** (composite modified with CuO 0.01%) **Group 2** (composite modified with CuO 0.005%)**41.91± 1.94MPa**. By being the control group has the maximum diametral tensile strength we concluded that both concentrations of nanoparticles have no effect on the mechanical properties of composite material.

According to **Altankhishig et al,** [24]who evaluated the effect of addition Zn and Cu oxides on tensile bond strength of nanocomposites to dentin. Where the results showed that there was in significant difference in tensile bond strength between the nanocomposite modified with CuO and the control group. And these results support our finding.

The results of compressive strength of the present study showed no significant difference between all test groups. The highest mean value was recorded in **Group 1** (control) **192.2±7.49MPa**, but within the experimental groups the highest mean value was for **Group 3** (composite modified with CuO 0.01%), **183.1±7.17 MPa**, then**Group2** (composite modified with CuO 0.005%), **180.7±7.99 MPa**. By being the control group has the maximum compressive strength we concluded that both concentrations of nanoparticles have no effect on the mechanical properties of composite material.

The results of color change of the present study showed no significant difference between all test groups. as the highest value was recorded by **Group3** (composite modified with CuO 0.01%) **15.99±0.70**, followed by **Group2** (composite modified with CuO 0.005%) **15.74±0.46**.

As, it was reported by **Fathy et al,** [19] the clinically acceptable value for color difference that cannot be visually perceivable in restorative materials is assumed to be $\Delta E^* \leqslant 3.3$. The average value recorded by our results was ≥ **3.5.** So, the used concentrations of both NPs caused change in color of the nano-filled composite, which decreases its esthetic properties.

The results of antibacterial activity of the present study revealed a significant difference between tested groups (p=0.00001). CuO was evaluated for its anti- bacterial effect by using both gram-negative (E.coli) and gram-positive (S.mutans) strains, where different concentrations were evaluated. CuO NPs had antibacterial activity against S,mutans and no activity against E.Coli.

The highest mean value was recorded in **Group3** [composite modified with CuO 0.01 (+ve)] **8.982±0.19 mm**, then **Group2**[composite modified with CuO 0.005 (+ve)] **7.623±0.21 mm.** No zone was seen in **Group2**[composite modified with CuO 0.005(-ve)], **Group3**[composite modified with CuO 0.01 (-ve)] and **Group1** (control group).

According to the results reported by **Dadi et al.** [25], regarding CuO NPs their results are the same as ours. As, they performed the experiment on St. aures (+ve), and E.coli (-ve) bacteria in order to evaluate the anti-bacterial activity of CuO NPs with range of concentrations from 0.1 mol/L to 1.5 mol/L. As their conclusion came as the following that CuO NPs are more effective against St. aures (+ve) than E.coli (-ve). These results are in consistence with our finding except, for E.coli results and this may be due to the lower concentration of CuO NPs used in the present study. Moreover, **Javadhesari et al,** [26] concluded the same results that CuO NPs affect gram (+ve) strains than gram (-ve) strains.

Being CuO NPs have antibacterial activity against S.mutans and no inhibition zone produced against E.coli. this may be justified with the direct relation between the concentrations of CuO and antibacterial activity against gram (-ve) strains, and also being E.coli has some way resistance against Cu toxicity[25,26].

V. CONCLUSIONS

Although, CuO NPs antibacterial activity against E.coli is concentration dependent, so larger concentrations may have activity against E.coli. However, it was concluded that use of CuO NPs doesn't affect mechanical properties and degree of conversion but, it increases its antibacterial activity against S. mutans and this prevents its future failure related to secondary caries and bacterial accumulation.

REFERENCES

[1]. Ferracane JL. Resin composite—state of the art. Dental materials. 2011;27(1):29-38.
[2]. Chen M-H. Update on dental nanocomposites. Journal of dental research. 2010;89(6):549-60.
[3]. Schmalz G, Hickel R, van Landuyt KL, Reichl F-X. Nanoparticles in dentistry. Dental Materials. 2017;33(11):1298-314.
[4]. Nassif M, El Askary F. Nanotechnology and nanoparticles in contemporary dental

adhesives. Nanobiomaterials in Clinical Dentistry: Elsevier; 2019. p. 163-98.

[5]. Kowalska A, Sokolowski J, Bociong K. The photoinitiators used in resin based dental composite—a review and future perspectives. Polymers. 2021;13(3):470.

[6]. AlKahtani RN. The implications and applications of nanotechnology in dentistry: A review. The Saudi dental journal. 2018;30(2):107-16.

[7]. Asghar M, Sajjad A, Hanif S, Ali JS, Ali Z, Zia M. Comparative analysis of synthesis, characterization, antimicrobial, antioxidant, and enzyme inhibition potential of roses petal based synthesized copper oxide nanoparticles. Materials Chemistry and Physics. 2022;278:125724.

[8]. Toodehzaeim MH, Zandi H, Meshkani H, Firouzabadi AH. The effect of CuO nanoparticles on antimicrobial effects and shear bond strength of orthodontic adhesives. Journal of Dentistry. 2018;19(1):1.

[9]. Harishchandra BD, Pappuswamy M, Antony P, Shama G, Pragatheesh A, Arumugam VA, et al. Copper nanoparticles: a review on synthesis, characterization and applications. Asian Pacific journal of cancer biology. 2020;5(4):201-10.

[10]. Nahar B, Chaity SB, Gafur MA, Hossain MZ. Synthesis of spherical copper oxide nanoparticles by chemical precipitation method and investigation of their photocatalytic and antibacterial activities. Journal of Nanomaterials. 2023;2023:1-10.

[11]. Boroumand S, Safari M, Shaabani E, Shirzad M, Faridi-Majidi R. Selenium nanoparticles: synthesis, characterization and study of their cytotoxicity, antioxidant and antibacterial activity. Materials Research Express. 2019;6(8):0850d8.

[12]. Akbari B, Tavandashti MP, Zandrahimi M. Particle size characterization of nanoparticles–a practical approach. Iranian Journal of Materials Science and Engineering. 2011;8(2):48-56.

[13]. Eid MM. Characterization of Nanoparticles by FTIR and FTIR-Microscopy. Handbook of Consumer Nanoproducts: Springer; 2022. p. 1-30.

[14]. Huseien GF. A review on concrete composites modified with nanoparticles. Journal of Composites Science. 2023;7(2):67.

[15]. Pérez-Mondragón AA, Cuevas-Suárez CE, González-López JA, Trejo-Carbajal N, Meléndez-Rodríguez M, Herrera-González AM. Preparation and evaluation of a BisGMA-free dental composite resin based on a novel trimethacrylate monomer. Dental Materials. 2020;36(4):542-50.

[16]. Gutiérrez MF, Bermudez J, Dávila-Sánchez A, Alegría-Acevedo LF, Méndez-Bauer L, Hernández M, et al. Zinc oxide and copper nanoparticles addition in universal adhesive systems improve interface stability on caries-affected dentin. Journal of the Mechanical Behavior of Biomedical Materials. 2019;100:103366.

[17]. Febrida R, Sahilah W, Adriana S, Djustiana N, Karlina E, Faza Y, et al. Diametral Tensile Strength and Reflectance Evaluation of Dental Composite Prototype Using Modified Dental Composite Procedures. Key Engineering Materials. 2020;829:100-7.

[18]. Saleem I, Rana NF, Tanweer T, Arif W, Shafique I, Alotaibi AS, et al. Effectiveness of Se/ZnO NPs in enhancing the antibacterial activity of resin-based dental composites. Materials. 2022;15(21):7827.

[19]. Fathy H, Haroun H, Riad M. The optical behavior of nano filled resin composite loaded with graphene oxide nanoparticles. BMC Oral Health. 2024;24(1):239.

[20]. Álvarez-Fernández E, Cancelo A, Díaz-Vega C, Capita R, Alonso-Calleja C. Antimicrobial resistance in E. coli isolates from conventionally and organically reared poultry: A comparison of agar disc diffusion and Sensi Test Gram-negative methods. Food Control. 2013;30(1):227-34.

[21]. Karikalan S, Mohankumar A. Antibiogram of Streptococcus mutans isolated from dental caries patients. International Journal of Medical and Health Research. 2016;2(3):79-83.

[22]. Zhang K, Zhang N, Weir MD, Reynolds MA, Bai Y, Xu HH. Bioactive dental composites and bonding agents having remineralizing and antibacterial characteristics. Dental Clinics. 2017;61(4):669-87.

[23]. Gutiérrez MF, Alegría-Acevedo LF, Méndez-Bauer L, Bermudez J, Dávila-Sánchez A, Buvinic S, et al. Biological,

mechanical and adhesive properties of universal adhesives containing zinc and copper nanoparticles. Journal of Dentistry. 2019;82:45-55.

[24]. Altankhishig B, Matsuda Y, Nagano-Takebe F, Okuyama K, Yamamoto H, Sakurai M, et al. Potential of fluoride-containing zinc oxide and copper oxide nanocomposites on dentin bonding ability. Nanomaterials. 2022;12(8):1291.

[25]. Dadi R, Azouani R, Traore M, Mielcarek C, Kanaev A. Antibacterial activity of ZnO and CuO nanoparticles against gram positive and gram negative strains. Materials Science and Engineering: C. 2019;104:109968.

[26]. Javadhesari SM, Alipour S, Mohammadnejad S, Akbarpour M. Antibacterial activity of ultra-small copper oxide (II) nanoparticles synthesized by mechanochemical processing against S. aureus and E. coli. Materials Science and Engineering: C. 2019;105:110011.