V. DISCUSSION

The results of the biologically and chemically synthesized copper oxide nanoparticles, characterization, toxicity analysis (lethal and sublethal), the impact of nanoparticles on biochemical, hematological and histological analysis on *Cyprinus carpio* and its antibacterial, antioxidant activity and biosensing property on melatonin hormone are discussed hereunder.

The *Hyptis suovelens* (L) poit leaf extracts contain alkaloids, flavonoids, saponins, tannins, phenols and phytosterols. However, the phlobatannins obtained from methanol extraction, terpenoids and anthraquinones are from ethanol extraction and glycosides from
ethanol and methanol extraction. Similarly, Shaikat Zeshan Hashib et al., (2012) obtained alkaloids, glycoside, saponins, tannins and flavonoids as major active constituents from ethanol extract of *Hyptis suaveolens* and phytochemicals present in the methanol extracts of *Hyptis suaveolens* (L). Kumkum Agarwal and Ranjana Varma, (2013) reported the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, glycoside, tannin, phenolic compounds, protein, amino acids, triterpenoids and steroids. Edeoga et al., (2006) reported that the aqueous extraction of *Hyptis suovelens* contains alkaloids, tannins, saponins, flavonoids and phenols. The methanol, ethanol and aqueous extraction of *Ocimum sanctum* contain alkaloids, flavinoids, saponins, phytosterols, tannins and glycosides. Bishnu Joshi et al., (2011) similarly reported the phytochemicals in the ethanol extract of *Ocimum sanctum* such as alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars.

Antibacterial activity of *Hyptis suovelens* (L) poit and *Ocimum sanctum* leaf extract from different solvents (aqueous, ethanol and methanol) showed 26.66, 22.66 and 19.33mm and 23.33,19.33 and 21mm respectively for both gram negative and gram positive bacteria (*E.coli, Staphylococcus aureus* and *Bacillus cereus*). Bishnu Joshi et al., (2011) reported that the aqueous and ethanolic extract of *Ocimum sanctum* subjected to antibacterial assay against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*. Mozhiyarasi and Anuradha (2016) reported that aqueous extract of *Hyptis suaveolens* (L.) poit showed 6mm diameter of inhibitory zone against both *Escherichia coli* and *Staphylococcus aureus*. In the case of ethanolic extract the inhibitory zone was 13mm and 10mm against *Escherichia coli, Staphylococcus aureus* respectively. The methonolic extract also showed significant inhibitory action (14mm) against *Escherichia coli* and 10mm against *Staphylococcus aureus*. Jeba Malar Renisheya Joy et al., (2012) studied that the antibacterial activity of ethanolic leaf extracts of *H. suaveolens* and reported the maximum zone of inhibition of 21 mm for *B.*
subtilis and 21 mm for E. coli. Pachkore et al., (2011) reported that the antimicrobial effect of aqueous and ethanol leaf extract of H. suaveolens using bacteria viz. Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. All the microbes tested are susceptible to ethanol extract with inhibition zone range of 12 – 29 mm. Rathnayaka, (2013) reported that the antibacterial activity of aqueous extract obtained from leaves of Ocimum sanctum against foodborne microbial pathogens, Escherichia coli and Staphylococcus aureus. A significantly higher antibacterial activity of Ocimum sanctum extract has shown against Staphylococcus aureus. Srinivas Naik et al., (2015) reported that the antibacterial activity of plant extract against gram-negative (Pseudomonas putida and Klebsiella pneumonia, E.coli) and gram-positive (Staphylococcus aureus and Bacillus subtilis) bacteria. Leaf extract showed good inhibition against the bacterial strains, observed antimicrobial activity could be explained by the fact that plant extract may attach to the surface of the cell membrane disturbing permeability and respiratory functions of the cell.

Antioxidant activity of Hyptis suovelens (L) poit and Ocimum sanctum leaf extracts in different solvents (aqueous ethanol and methanol) extraction are 94.99, 89.99, 96.22% and 95.33, 92.43, 94.09 % respectively in 300 µl of extracts. Kumkum Agarwal and Ranjana Varma, (2013) reported that the antioxidant activity has a potent ability of 69.46% at 100 µg/ml concentration alcohol extract of Hyptis suaveolens L. Poit. Nithya Narayanaswamy and Balakrishnan, (2011) reported that the DPPH scavenging potential of the aqueous extracts and ethanolic extracts of medicinal plants (including Hyptis suovelens) and the inhibition is in the range of 38 % -95%. Gavani and Paarakh, (2008) reported the antioxidant activity of methanol extraction of leaf of Hyptis suovelens Poit. and evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity using gallic acid and butylated hydroxyanisole (BHA) as reference standards. Prasad et al., (2012) reported that the antioxidant activity was measured by reducing power assay, 1-1-diphenyl-2-picrylhydrazyl
(DPPH) assay and Thiobarbituric acid (TBA) which showed positive in methanolic extracts of *Ocimum* sp. Sailaja Inampudi and Shaker Ivvala Anand, (2010) reported that *Ocimum basicilicum* seemed to be a better source of antioxidant compounds followed by *Ocimum sanctum* in DPPH assay. Balaji *et al.*, (2016) reported that the *Ocimum tenuiflorum* leaf and stem extracts for free radical scavenging properties using BHT (Butylated hydroxyl toluene) and ascorbic acid as standard antioxidant. The total phenolic content was estimated in both extracts, leaf extract showing more activity than the stem. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was assayed, leaf showing 71% higher activity than stem.

The retention times of phytochemicals in the methanol extract of *Hyptis suaveolens* (L) poit were identified as O - Methylisourea, Diethyl benzene 1,2 dicarboxylate, (2E,7R, 11R) ,7,11,15 tetramethyl 2 hexadecen 1ol, 7Z,10Z,13Z,16Z docosatetraenoic,(4bS,8aS) 4b, 8,8 Trimethyl 2 propan 2 yl 5,6,7,8a,9,10 hexahydrophenanthren 3ol, acid, Pentyl benzoate and Methyl-3-Bromo -1- Adamantaneacetate was identified by Gas Chromatography- Mass Spectrum (GC-MS). Joseph Joselin and Jeeva Solomon, (2016) reported that the GC-MS analysis led to the identification of a number of compounds from the GC fractions of the ethanolic extract of *H. Suaveolens* using PerkinElmer GC-MS, among the 30 phytochemical compounds, 5,5-Dimethylimidazolidin-2,4-diamine (20.35%) was found to be the major compound. Mozhiyarasi and Anuradha, (2016) reported that the phyto constituent of ethanolic extract of *Hyptis suaveolens* (L) poit was identified using GC-MS. Twenty five compounds identified includes Eucalyptol (C_{10}H_{18}O)0.44%, Bicyclo [4.1.0] 3 heptene, 2 isopropenyl 5 isopropyl 7,7 dimethyl (C_{15}H_{24}), 0.07, Cyclobutaneacetonitrile, 1 methyl 2 (1methyl ethenyl) (C_{10}H_{15}N) 0.05, Bicyclo [7.2.0] undec 4 ene, 4,11,11 trimethyl 8 methylene, [1R (1R,4Z,9S)] (C_{15}H_{24}) 2.44 1,3,6,10 Dodecatetraene, 3,7,11-trimethyl-, (Z,E)- (C_{15}H_{24}) 0.30, 1,3,7-Octatriene, 3,7-dimethyl- (C_{10}H_{16}) 0.10, Adamantane, 1-(2-bromoethenyl)-
(C_{12}H_{17}Br) 0.15, 1,9-Decadiyne (C_{10}H_{14}) and 0.03, Squalene (C_{30}H_{50}) 6.47. Ismail Mohamed and Sheik Jahabar Ali, (2017) reported that the *Hyptis suaveolens* (L.) poit methanol extract obtained by solvent extraction were analyzed by gas chromatography-mass spectroscopy (GC-MS) and thirty-five components were identified in the leaf extract. The major components were Phenanthrene ethanol (26.19%), Podocarp (8.68%), 9-Octadecynoic acid (8.16%), Palustic acid (8.05%), n-hexadecanoic acid (7.23%), bicycle (3.1.1) hept-2-ene, 2, 2’(1, 2-ethanediyl) bis(6, 6-dimethyl (6.75%), 7-isopropyl-1, 1, 4a- trimethyl-1, 2, 3, 4, 4a, 9, 10, 10a-octahydrophenanthrene (6.36%), benzaldehyde, 2-hydroxy6-methyl (6.02%), 3, 7, 11, 15-tetramethyl-2-hexadecen-ol (4.61%). The compositions of Methanol extract varied qualitatively and quantitatively.

Based on antibacterial, antioxidant test, *H. suaveolens* (L) poit extraction form methanol solvents were used for the synthesis of CuO Nps by biological method. The formations of CuO Nps are easily discernible by color change. The UV - Vis analysis of biologically and chemically synthesized CuO Nps shows the sharp peaks at 249nm and 264nm, this peak indicates the formation of CuO NPs. Vijay Kumar *et al.*, (2015) reported that the biologically synthesized CuO nanoparticles by using *Phyllanthus amarus* leaf extract and UV-spectra indicated that the CuO SPR bands are obtained around 285 nm with the absorption edge at 436 nm. Similarly, Sutradhar Prasanta *et al.*, (2014) reported that strong absorption peak at 269 nm for CuO Nps synthesized by using tea leaf and coffee extraction and between 200 – 300nm for CuO Nps synthesized from *C. papaya* leaves extract. Raja Naika *et al.*, (2015) reported that the synthesis of copper oxide nanoparticles (CuO Nps) using *Gloriosa superba* L. plant extract and UV–vis spectra, the formed CuO Nps dispersed in water exhibiting the maximum absorption peaks at about 380 nm. Jayalakshmi and Yogamoorthi, (2014) reported that the synthesized copper oxide particles are subjected to UV- spectroscopic analysis and obtained a single peak but broad at 263nm indicating the
presence of oxides of copper metal. Gopinath et al., (2014) reported the characterization of copper nanoparticles from Nerium oleander leaf aqueous extract by UV-Spectrophotometer from the range 250-450 nm. Pulicherla Yugandhar et al., (2018) reported the synthesis of copper oxide nanoparticles (CuO NPs) using fruit extract of Syzygium alternifolium, peak manifested at 285 nm in UV–Vis analysis confirms the synthesis of CuO NPs.

In biologically and chemically synthesized copper oxide nanoparticles, FT-IR peaks correspond to O-H stretch phenolic compounds, N-H stretch of amines, O-H stretch of Carboxylic group, N-H bending of amines, C-H stretch of aliphatic amines, C-Cl stretch of alkaloids, C-H bending of alkanes and these peaks are similarly reported by Abbas Eslami et al., (2017). Riya Lily and George Mary, (2015) observed a peak at C-H bending of alkanes (622cm⁻¹) indicate the formation of cuprous oxide nanoparticles synthesized by using Camellia sinensis (Green tea) plant extract. The bands around 3450, 1600 and 2350 cm⁻¹ show the presence of O-H, C=C and C=O stretching of hydroxyl groups, alkenes and presence of alkanes respectively, a strong band at 1100 cm⁻¹, and 529, 350 cm⁻¹ confirming the formation of highly pure CuO nanoparticles. Vijay Kumar et al., (2015) reported spectral peaks proposing the occurrence of bands relevant to amide NAH stretching (3444 cm⁻¹), alkane CAH stretching (2926 cm⁻¹) anhydride C-O bending (1880 cm⁻¹) and C-O stretching (1087 cm⁻¹). Azom et al., (2012) reported that the existence of band at 473 cm⁻¹ recognized as O-H stretch of Carboxylic group, confirming the formation of copper oxide nanoparticles.

The crystalline structure and size of biologically and chemically synthesized CuO nanoparticles were analyzed by using XRD analysis, a series of diffraction peaks and plane values shows hexagonal phase (JCPDS-80-1916) and 67nm and 65nm in size respectively. Similarly, Srivastava Sanjay et al., (2013) reported that the lattice parameters of unit cell of CuO are found as 4.691 Å, b = 3.432 Å, c = 5.138 Å and the peak positions with 2theta
values of 29.4, 36.8, 42.1, 61.9 and 77.6 are indexed with 110, 111, 200, 220 and 222 planes of biologically synthesized CuO Nps by using alga (*Bifurcaria bifurcata*) extract. Abboud *et al.*, (2014) reported that the CuO Nps synthesized by the hydrothermal method is indexed as monoclinic phased CuO NPs by comparison with a Joint committee on Powder Diffraction Standards (JCPDS) card files No. 80-1916. Abbas Eslami *et al.*, (2017) reported that the XRD patterns of all the diffraction peaks are in good agreement with the standard diffraction data for CuO (JCPDS 45-0937), no characteristic peaks were observed for other oxides (such as Cu2O or Cu2O3) and the average crystallite size estimated by Debye-Scherer equation was about 71 nm.

The SEM image of biologically and chemically synthesized CuO Nps clearly shows that the particles are small and uniform size, almost spherical in nature which is free from agglomeration that indicates the spherical and rectangular shape of synthesized copper oxide nanoparticles. Gultekin Demet Demirci *et al.*, (2017) reported that the copper nanoparticle was synthesized by using the water extract of Erzincan Cimin grape (*Vitis vinifera cv*). Nithya *et al.*, (2014) and Hemalatha and Makeswari, (2017) reported the synthesis of CuO nanoparticles from *Aloe barbadensis* has a well defined morphology and are nearly spherical in shape. Saranyaadevi *et al.*, (2014) reported the synthesis of Cu NPs by the plant extract of *Capparis zeylanica*. Synthesized Cu Nps were obtained by Scanning Electron Microscopy (SEM) analysis and shown that spherical and relatively uniform shape of the copper nanoparticles was confirmed in the range of 60-100nm. Javad Karimi and Sasan Mohsenzadeh, (2013) reported that the formation of copper nanoparticles from the *Aloe vera* flowers extract as well as their morphological dimensions in the SEM study demonstrated that the average size was 40 nm and was spherical in shape. Suresh *et al.*, (2014) reported that the SEM image of tea decoction stabilized copper nanoparticles prepared from copper sulfate salt and the image show clear spherical morphology with an average particle size around 5 nm.
Mina Sorbiun et al., (2018) reported the green synthesis of highly crystalline CuO nanoparticles (NPs) by oak fruit hull (Jaft) as reducing and stabilizing agent and observed that most of the CuO nanoparticles are in nanometre scale and are mostly of quasi-spherical shape.

The elemental composition of the biological and chemical synthesized CuO Nps was identified as spherical in nature and sizes were 82 % and 67% by an Energy Dispersive X-ray Spectroscopy. Alwin David et al., (2017) reported the presence of copper and oxygen signal peaks in the EDX spectrum confirms CuO Nps which are synthesized by using Momordica charantia leaf extract. Vanathi et al., (2016) also reported that Aloe barbadensis mediated copper oxide nanoparticles were spherical in nature and elemental compositions were confirmed by EDX. Ayesha Khan et al., (2016) reported that the synthesis of Cu Nps by chemical reduction method and ascorbic acid as reducing agent at low temperature (80°C). EDX spectroscopy is applied to quantify the elemental composition of the synthesized Cu nanoparticles in 83.75 %. Pulicherla Yugandhar et al., (2018) synthesized copper oxide nanoparticles (CuO NPs) using fruit extract of Syzygium alternifolium and the EDX analysis of nanoparticles showed 34.32 and 31.54% of copper oxide.

The physico-chemical parameters of tap water such as pH, temperature, dissolved oxygen, chloride, total hardness and dissolved carbon dioxide were 6.8, 24.5°C, 0.8 mg/L 71mg/l, 320 mg/l and 2.2 mg/l respectively. Sharmila and Kavitha (2017) reported similar physico-chemical parameters of the test medium (Temperature - 23 ± 5°C, dissolved oxygen- 6.5 ± 0.15 mg/l, Salinity-2.7 ± 1 ppt and pH -7.0 ± 1). Bhatnagar Anita et al., (2016) reported that the physico-chemical properties of test water such as temperature, pH, dissolved oxygen and total hardness was 26.3±0.36°C, 7.3 to 7.5 ± 0.03, 5.64±0.03 mg/L⁻¹, 350 ± 1.15 to 398 ± 0.06 respectively. Ahmad Maqbool and Ahmad S. Bajahlan, (2009) reported the physico – chemical parameter of tap water as pH, 8.37 and Ajani and
Akpolin.(2010) reported that the tap water quality parameters such as temperature, pH, dissolved oxygen are 28.5° C, 6.5, 5.5 to 6.5 mg/l respectively.

Vidya and Chitra, (2017) reported that nanotoxicological studies primarily focus to investigate the effects of Nps based on the median lethal concentration, as it can directly measure the toxicity of compounds on the exposed organism. The median lethal concentrations of both biologically and chemically synthesized CuO NPs are 23.41, 1.84 mg/L, respectively for *Cyprinus carpio* to a period of 14 days. This indicates chemically synthesized CuO Nps is more toxic than that of biologically synthesized CuO nanoparticles. Vajargah Mohammad Forouhar *et al.*, (2018) reported the toxicity of copper oxide NPs (NPs-CuO) on common carp (*Cyprinus carpio*) exposed to 0, 10, 20, 30, 40, 60, 80, 100, 150, and 200 mg/l for 96 hrs. Safina Kousar and Muhammad Javed, (2012) reported the acute toxicity of copper (Cu) on four fish species viz. *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*.

Based on median lethal concentration values of biologically and chemically synthesized CuO Nps, five different concentrations such as 0 (control), 0.23, 0.31, 0.46, 0.93ppm and 0.018, 0.024, 0.036, 0.072 ppm respectively were selected and fish were exposed for 14 days. Similarly, Hedayati *et al.*, (2013) and Khadijah Kadhem Haraib, (2012) exposed common carp fish to a high concentration of CuSO4 (0.49 mg/l) and a low concentration (0.09 mg/l) for the period of 24, 48, and 96 hrs by static toxicity assessment and the fish samples were analyzed before and after the treatments.

The protein, carbohydrate and lipid in muscle, gill, and liver of *Cyprinus carpio* decreased as the concentration of biologically and chemically synthesized CuO Nps when compared to the control group. Sevcikova *et al.*, (2016) and Vutukuru *et al.*, (2013) reported a decreased total glycogen content of the fish tissues viz., gill, muscle when exposed to CuO and TiO₂ Nps in Common carp and Zebra fish respectively. Mehibeen javad and Nazura
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usmani, (2014) reported that the decreased total protein, albumin and globulin level when exposed to Cu Nps in Cyprinus carpio fish tissue viz., liver and muscle. Abdel-Tawwab Mohsen et al., (2013) reported that the total protein and lipid decreased significantly by increasing Zn Nps concentrations and exposure period. This decrease may be due to Zn exposure which causes significant alteration in the protein secondary structure by decreasing the α-helix and increasing the β-sheet content of the gill tissues of carp Rohu (Labeo rohita).

Similarly, Rajeshwari and Sevarkodyione, (2017) reported that the carbohydrate, lipids and protein content in Cyprinus carpio fish tissue viz., muscle and liver were exposed to sublethal concentrations of cadmium Nps for various exposure periods.

Hematological parameters can be useful for the measurement of physiological disturbances in stressed fish and thus used as a reliable indicator for toxicological research, environmental monitoring and as indicators of disease and stress (Shaluei et al., 2013). In this study, hematological parameters such as WBC, polymorphic, neutrophils, lymphocytes, eosinophil, RBC and hemoglobin levels in Cyprinus carpio decreased significantly (P < 0.05) with increasing concentration of biologically and chemically synthesized CuO Nps. Similar results are also reported in fish Cyprinus carpio exposed to sublethal concentration of ammonia for 35 days (Thangam et al., 2014), in rainbow trout (Oncorhynchus mykiss) exposed to CuSO₄ (Shaw J. Benjamin et al., 2012). Mayilathal and Thamizhselvi, (2014) reported that the blood parameters like total RBC count, WBC count, hemoglobin content and hematocrit in the blood of C. carpio exposed to LC₅₀ value of lead nitrate (4.45 ppt) are decreased with exposure periods. The decrease in RBC count, Hb and Hct levels may act as indicators of acute anemia. John et al., (2007) and Lavanya and Veerappan, (2011) suggested that in toxicological experiments the decrease in RBC count, Hb and Hct levels, lysing of RBC due to toxicant stress may also lead to a reduction in Hb and Hct values in the fish, it leads to alteration in the selective permeability of the membrane. Kori-Siakpere and
Oghoghene, (2008) reported that an increase in the number of white blood cells is a normal reaction of fish to substances that alter their normal physiological processes. But in this present study, WBC count was significantly decreased with increase in the concentration of CuO Nps, it may due to toxic effect of nanoparticles and stress caused on the cell production activity of the spleen.

Histopathological analysis of gill and liver of C. carpio exposed to biologically and chemically synthesized CuO Nps exhibit gill proliferation of bronchial chloride cells that leads to lamellae fusion and formation of an aneurysm. Gill is one of the most important organ in the body of fish, plays several critical roles in the body in the form of osmoregulation, respiratory gas exchange, and body fluid permeability balance (Chidambaram Jeyaseelan et al., 2014). The concentration of CuO Nps increases the gill damage such as hyperplasia, edema, curvature, shortening and fusion of gill lamellae, aneurism and necrosis. Hence, gill is an organ with the large superficial area of the epithelium and direct contact between this organ and water, it is more susceptible to chemical pollutant effects in aquatic systems (Melika, 2014) and similar gill damage was also reported with CuO Nps exposure to common carp Cyprinus carpio. (Al-Bairuty et al., 2013). Mansouri Borhan et al., (2015) reported that the two main changes in fish under acute and chronic exposure to toxic substances such as NPs are clubbed tips and curvature of the lamellar epithelium. Under this condition, the epithelium layer of secondary lamella becomes edema and the presence of chemicals reduces the surface area, resulting reduction of gas exchange in the gills. Edema is a common alteration in gills of fish exposed to NPs. According to Altinok and Capkin, (2007) and Dabrowska et al., (2012), the hepatic histopathological lesions are often evaluated in toxicological studies and used as markers of environmental pollution. Liver tissue in control showed normal hepatocytes and CuO Nps treated fish had congestive enlargement of liposome, abnormal arrangement of muscle
bundles, vacuolization, and inflammation. The liver shows a high potential for enzymatic degradation of toxic compounds, it may be due to high concentrations of nanoparticles. Similarly, Song et al., (2015) reported that the rainbow trout exposed to 1 mg Cu/L and 0.5 mg Cu/L induced alterations in the gill tissues, which included both proliferation and detachment of epithelial cells at the base of secondary lamellae, fusion of secondary lamellae and proliferation of epithelial cells in the gills. Similar changes observed by HAO Linhua et al., (2009), the liver of the Juvenile carp (Cyprinus carp) exposed to TiO$_2$NPs showed characteristic cytoplasm vacuolation and apoptosome including necrotic cell bodies and nuclear fragments which appeared to be apoptotic bodies, and a few foci of lipidosis with minor fatty change, mainly at high TiO$_2$-Nps concentrations. Shaw and Handy, (2011) reported that fish exposed to Cu NPs displayed blood accumulation and increase in sinusoid space, which is an indication of liver damage and Cu-NPs even to doses lower than LC$_{50}$ showed a pronounced increase in the number of pyknotic nucleus indicating dead nuclei that may progress to tissue necrosis where as on higher dose exposure displayed accumulation of lipid droplet in the hepatocytes or forming vacuole and cellular swelling with a clear cytoplasm due to the presence of small vacuoles, with indistinct shape. This also limits the normal position of the nucleus. In this respect, histological analysis endorsed the altered levels of antioxidant enzymes in the common carp liver (Gupta Yugantak Raj et al., 2016).

The result of the antibacterial activity of biologically and chemically synthesized CuO Nps against gram negative (*E.coli*) and gram positive bacteria (*Staphylococcus aureus, Bacillus cereus*). The results indicated that biosynthesized CuO Nps showed the effective antibacterial activity (22, 20, 18 mm) both in gram negative and positive bacteria than chemically synthesized CuO Nps (19, 17, 15.89 mm). Raja Naika et al., (2015) reported the synthesis of copper oxide nanoparticles (CuO Nps) using *Gloriosa superba* L. plant extract and exhibited significant antibacterial activity against four bacterial strains, i.e., Gram –ve
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K. aerogenes, E. coli, and P. desmolyticum, Gram +ve bacteria S. aureus. Among them, K. aerogenes and E. coli show a significant zone of inhibition to CuO Nps compared to the positive control. Amr et al., (2016) also reported the potential bactericidal activity of CuO NPs on various carcinogenic bacteria. From this study, the result proves that nano copper oxide had a good inhibitory effect on the bacterial species. Ivan Sondi and Branka Salopek-Sondi (2004) reported that the silver nanoparticles is used as antimicrobial agent against E. coli on agar plates and in liquid LB medium.

DPPH provides an easy and rapid method of estimating antioxidant activity. In the biologically and chemically synthesized of CuO Nps, DPPH scavenging activity is 72.56% and 69.04 %, respectively in the concentration of 300 µl CuO Nps, while in control, ascorbic acid was 97.08%. From the result, the best scavenging activity is shown in biologically synthesized CuO Nps than chemically synthesized CuO Nps. Ghosh Sougata et al., (2015) reported that the D. bulbifera tuber extract mediated bioreduction is a most rapid route to synthesize novel CuNPs with promising antidiabetic and antioxidant properties. Phull Abdul-Rehman et al., (2016) reported that green synthesized nanoparticles showed the enhanced antioxidant properties compared to the crude extract analysis. El-Basuini Mohammed Fouad et al., (2016) reported that the Cu Nps has the capacity to produce reactive oxygen species (ROS) by interacting with subcellular structures, leading to the production of oxyradicals that activate antioxidant systems. Renata Dobrucka, (2018) reported that the antioxidant properties of CuO nanoparticles synthesized using the extract of G. herba the value of the parameter was 4.12 µg/ml. The result indicate that the CuO nanoparticles showed high antioxidant activity.

From the application point of view, chemical sensing performance of biologically and chemically synthesized CuO Nps was investigated by various chromatographic and spectroscopic techniques. Melatonin hormones were detected by electrochemical properties at
pH 12. This result, clearly explains that the biologically synthesized CuO Nps are highly potential than chemically synthesized CuO Nps in sensing melatonin hormone. Hong-Ying Yu et al., (2013) and Yadav Pankaj et al., (2017) similarly reported the electrochemical performance of the CuO- graphene modified glassy carbon electrode for glucose sensing by cyclic voltametry (CV) and amperometric I-t Curve. The CuO graphene modified electrode shows good electro catalytic activity towards the oxidation of glucose in 0.1M NaOH solution which highlighted by simple preparation process for highly sensitive and selective DA sensor along with an indepth characterization. Deng Jianmian et al., (2018) reported that the Plectranthus amboinicus leaf extract was employed for the biosynthesis of ZnO nanoparticles (NPs) and a novel electrochemical sensor was fabricated based on the ZnO NPs coated glassy carbon electrode (GCE) and also used for the detection of the organic pollutant hydrazine.

Sathish Reddy et al., (2012) reported that the different shaped CuO nanoparticles were synthesized using cetyl trimethyl ammonium bromide (CTAB) and sodium dodecyl sulfate (SDS) in a co-precipitation method. The prepared CuO nanoparticles were used for the preparation of modified carbon-paste electrodes (MCPE) for the electrochemical detection of dopamine (DA) at pH 6.0. Xue Wang et al., (2010) reported that the synthesized CuO Nps have been used to construct nonenzymatic glucose sensors, and sensors exhibited highly enhanced electrocatalysis towards glucose oxidation compared with that of a bare graphite electrode. Though the CuO flowers/G electrode can produce a high sensitivity of 709.52A/mM with the initial injection of 0.5mM glucose and a low detection limit of 4mM, due to its slow electron transfer. Ji Ping Shi et al., (2010) reported that the electro catalytic reduction of hydrogen peroxide in alkaline medium at a carbon ionic liquid conductor changed with copper oxide nanoparticles. The combination of the good conductivity of the ionic liquid and the high catalytic activity of the nanoparticles resulted in an electrode
with attractive properties for the determination of hydrogen peroxide. The linear range for the
determination of hydrogen peroxide is from 1.0 μM to 2.5 mM, the detection limit is 0.5 μM
high stability, sensitivity, selectivity and reproducibility.

Ai Ling Hu et al.,(2014) reported that the fluorescent hydrogen peroxide was
developed based on the peroxidase activity of cupric oxide nanoparticles. Cu Nps completely
catalyzed the hydrogen peroxide into hydroxyl radicals. The terephthalic acid was oxidized
by hydroxyl radical to form a highly fluorescent product. The linear arrangement of hydrogen
peroxide estimated to be $5.0 \times 10^{-6}$ to $2.0 \times 10^{-4}$M with a detection limit $3.4 \times 10^{-7}$M and
sensitive assays of glucose and lactate with detection limits of $10 \times 10^{-6}$ and $4.5 \times 10^{-8}$M.
Hong Lei et al., (2013) reported that chemiluminescent cholesterol sensor was constructed
based upon the peroxidase with good selectivity and enhanced sensitivity like the activity of
cupric oxide nano particles. Cupric oxide nanoparticles catalyze the oxidation of luminal
hydrogen peroxide, which was produced by reaction of cholesterol oxidase. Therefore,
the oxidation of cholesterol into the chemiluminescence of luminal by combining these two
reactions. The CL intensity was proportional to the concentration of cholesterol over the
range of 0.625 – 12.5 mM and a detection limit was 0.17Mm.