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Tamra Bhasma (Copper Oxide Nanoparticles) as the Ancient Science of Biomedicines

Himani Panwar★ and Ramesh Chandra Dubey★★
★Deptt. of Botany and Microbiology, Gurukula Kangri (Deemed to be University), Haridwar-249404, UK, Indian

Recent advances in science and technology, particularly in nanotechnology have greatly expanded the range of uses for metal oxide nanoparticles across industries, research institutions and scientific disciplines. Copper oxide nanoparticles (CuO NPs) are a specific kind of nanoparticles that have gained significant attention among metal oxide nanoparticles because of their diverse features and potential uses in several fields, especially biomedical sciences and nanomedicine. Humans have been using copper since the prehistoric era. In Ayurveda, it is incredibly valuable and widely used in many ways to treat a wide range of ailments. *Tamra* (copper) has been utilized for ages in daily life as medicine and vessels. Since the *Charaka Samhita* (1500 BC), the idea of reducing the size of metal particles has been described. This has allowed nanotechnology to create larger structures with novel molecular organisation. With the introduction of *Rasashastra* in the Middle Ages, *Tamra Bhasma* played a significant role in Ayurvedic medicine. It is the preferred medication for treating a wide range of illnesses, including *Kushtha* (skin conditions), *Yakritvikara* (hepatic problems) and *Udara* (ascitis). Numerous studies have been conducted on it recently. *Tamra Bhasma*, which is derived from metallic copper, is advised for a variety of liver and spleen conditions, dropsy, abdominal pain, heart disease, colitis, tumours, anaemia, appetite loss, tuberculosis and vision issues. This article provides an overview of the copper oxide nanoparticles and their potential uses in biomedicine.

Introduction

Nanotechnology is a recent technological advancement with widespread applications across various industries for creating cost-effective and environmentally friendly products. Modern drugs now utilize nanoparticles made from polymers and metals, exhibiting efficacy against conditions like cancer and microbial pathogens. Embracing nanometer-sized drugs enhances their performance in various dosage forms. In the pharmaceutical field, Nanotechnology has given rise to developments in nanomedicine, tissue engineering, nanorobots, biosensors, biomarkers and more, offering opportunities to enhance materials and medical devices. Nanotechnology's flexibility allows the creation of various forms of nanomedicines like liposomes, dendrimers, nanoparticles and nanocrystals to serve diverse biomedical applications.

★ Residence : House no. 67, Gali no. 6, Bhagirathi vihar part 2, Lakshar road Near jhadu wala, Haridwar-249404, Uttarakhand, Email : himanipanwar1012@gmail.com

Nanoparticles are at least one dimension in size and range from 1 to 100 nm. Nano-drug delivery systems significantly improve the pharmacokinetics and biodistribution of active ingredients by targeting specific sites, thereby enhancing efficiency, bioavailability and reducing drug toxicity (Sahu et al., 2021). Remarkably, the roots of using metals and minerals for medicinal purposes trace back to the eighth century AD in India, where alchemist Nagarjuna introduced the use of substances like gold, silver, copper, mica, pyrites and mercury (Conde et al., 2014).

Ayurveda, the traditional Indian medicinal system has a branch known as *RasaShastra*, dealing with herbo-metallic preparations. Practitioners of both ancient and modern approaches are currently challenged to standardize the synthesis process and conduct thorough scientific investigations to ensure the safety and purity of these metal-based bhasmas. The Ayurvedic *Bhasmas*, used for centuries in the form of nanotechnology, exhibit properties such as "*Rasayana*" (immune modulation and antiaging) and "*Yogavahi*" (drug carry and targeting drug delivery) (Othayoth et al., 2014). These *Bhasmas*, coated on nano-tablets, have demonstrated anticancer activity, showcasing the surprising knowledge of nanoscience and technology within the 5,000-year-old Indian medical system. The ancient medicinal system of Ayurveda, practiced in India for centuries, involves the transformation of metals into mixed oxides known as "*Bhasma*." Copper has incredible centrality in Ayurveda and is broadly utilized in various manners to fix numerous maladies. *Tamra Bhasma* is one of the popular Ayurvedic medications based on metal oxides. The concept of reducing the particle size of metals has persisted since Charaka Samhita (1500 BC), enabling nanotechnology to produce larger structures with new molecular organization. The advent of nanoparticles is nothing short of a miracle, offering transformative products and technologies in medicine. Ayurvedic nanomaterials possess unique physicochemical properties such as biocompatibility and ease of surface functionalization (Pal and Gurjar, 2017).

In Charaka Samhita, *Tamra* is delineated as one of the six metals (Charaka Sutrasthana 1/71). Tamra is recommended for treating various ailments; its powder is prescribed for internal use as *Rasayana* (rejuvenation) and for managing diseases (Charaka Chikitasthana 1-3/46). A wide range of diseases, including *Krimi* (worms), *Sthaulya* (stoutness), *Arsha* (hemorrhoids/piles), *Ksaya* (seizure/fit), *Pandu* (iron deficiency anaemia), *Kusta* (leprosy), *Swasa* (asthma), *Kasa* (cough), *Amlapitta* (gastritis/acidity), *Sotha* (oedema), *Sula* (chest pain), *Yakrit Roga* (liver diseases), etc. can be effectively treated with different formulations of "*Tamra*" (copper) as mentioned in *Charaka Samhita*.

मुक्ताप्रवालवैदूर्यशंखस्फटिकमंजनम्।
ससारगंधकाचार्कसूक्ष्मैलालवणद्वयम् ।।
ताम्रायोरजसी रूप्यं ससौगंधिकसीसकम्।

जातीफलं शणाद~बीजमपामार्गस्य तंडुला: ।।
एषां पाणितलं चूर्ण तुल्यानां क्षौद्रसर्पिष ।
हिक्कां श्वासं च कासं च लीढमाशु नियच्छति ।।
अंजनात्तिमिरं काचं नीलिकां पुष्पकं तम: ।
मल्यं कंडूमभिष्यंदमर्म चैव प्रणाशयेत् ।।

The above-mentioned shloka from *Charak Samhita* (Vol. 2) also emphasizes the use of *Tamra* (Copper) in treating various diseases. Furthermore, the various forms of copper that are found to be most suitable for medical use are those that exhibit the distinctive metallic sheen (*Snigdham*), are soft (*Mridulam*), have a bright reddish colour (*Shonam*), have high tensile strength (*Ghanaghata Ksamam*), are heavy (*Guru*) and are pure (*Nirvikaram*). The employment of *Tamra* vessels is advocated for addressing skin conditions like *Sidhma* and *Kilasa* (Charaka Chikitasthana 7/117-118). Additionally, its *Anjana* (collyrium) is employed for addressing *Abhishyanda* and *Timira* (Charaka Chikitasthana17/125). *Tamra*, in conjunction with other minerals and metals such as gold, is employed for managing conditions like *Visarpa* and *Gulma* (Charaka Chikitasthana 21/131). Various formulations like *Anjana*, *Shankha Varti* and *Drishtiprada Varti* are prepared using *Tamra* powder combined with other substances to treat all eye ailments and enhance the patient's vision. The *Tamra* pot is highly recommended for preparing *Anjana* (Charaka Chikitasthana 26/254-255). *Basti Netra* (nozzles) and *Jihva nirlekhaka* (tongue cleaner) are purportedly crafted from *Tamra* and other metals. *Rigveda* references the term *Ayas*, possibly denoting copper and Ayurveda emphasizes the use of copper ornaments, denoted as *Ayas* and *Shyamam* (*Atharvaveda* 1/34/6, 1/34/7, 20/8/31).

CuO NPs illustrated remarkable anticancer, antibacterial, antifungal and biosensor activity (Fig.1) in addition to the previously mentioned use, rendering them an attractive tool for biomedical applications.

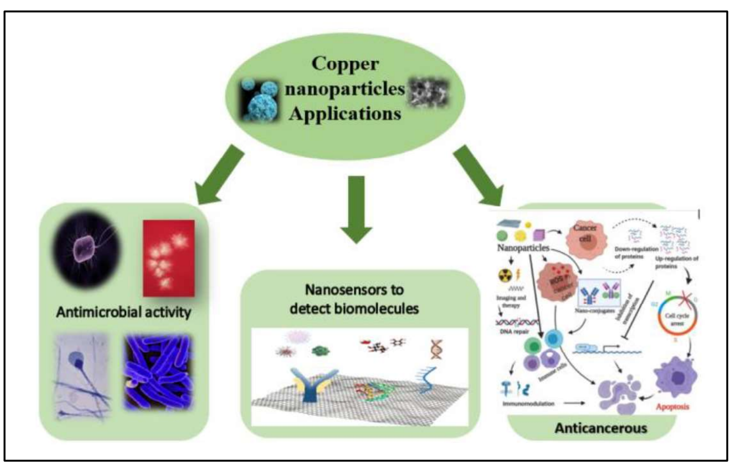

Fig. 1: Applications of Copper nanoparticles in the Biomedical field.

Antibacterial property

Nano-based therapies have been used in the last few years for the development of new medications as well as the diagnosis and treatment of illnesses. When tested against various pathogenic bacterial strains, the antibacterial properties of nanoparticles demonstrated notable results. CuO-NPs are extremely hazardous to most human infections, according to literature research because of their distinct morphologies, small size and biocompatibility, biofabricated copper oxide nanoparticles have garnered significant interest as antibacterial agents to treat a variety of human pathogenic bacteria. Additionally, the green-synthesized copper oxide nanoparticles have strong antibacterial properties against Gram-positive and Gram-negative bacterial strains. Such nanoparticles used for disease treatment are referred to as "Nanoceuticals". Naika et al. (2015) investigated the antibacterial properties of copper oxide nanoparticles made from *Gloriosa superba* L. extract against strains of Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella aerogenes, P. desmolyticum, Escherichia coli*). Using an extract from *Acanthospermum hispidum* L., Pansambal et al. (2017) reported synthesizing copper oxide nanoparticles and assessed their antibacterial properties. The outcomes showed that the green-synthesized copper oxide nanoparticles have strong antibacterial properties against *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes* and *S.aureus.* According to Sathiyavimal et al. (2022), Copper oxide nanoparticles synthesized from *Abutilon indicum* leaf extract was found to be effective against human pathogenic bacteria such as *E.coli, Salmonella typhi, Bacillus subtilis* and *S.aureus* and showed highest bactericidal activity. With a diameter of inhibition zones ranging from 25 to 32 nm, the maximum antibacterial activity of rod CuO NPs fabricated using *Momordica charantia* aqueous fruit extract was observed against *S. aureus, S. epidermidis, Streptococcus mutans, S. pyogenes, S. viridans, B. cereus, Corynebacterium xerosis, E. coli, K. pneumonia, P. aeruginosa* and *Proteus vulgaris* at 250 μg mL^{-1} (Eid et al., 2023). Cu/CuO NPs have stronger activity than bulk compounds, even though copper metals (Cu^{2+}) have antibacterial capabilities and are recognised by the US-EPA (US Environmental Protection Agency) as a safe antimicrobial agent (Risks, 2007).

Antifungal activity

In the history of medicine, fungal diseases have grown to be serious health problems, particularly for those with weakened immune systems, such as those with AIDS, or for cancer patients getting chemical treatment. For potential use in medicine, copper oxide nanoparticles antifungal activity has been studied. These nanoparticles may be able to treat fungal infections. In contrast to their antibacterial activity, the antifungal properties of copper oxide nanoparticles have received less research attention. In a study by Garcia-Marin et al. (2022), CuO NPs synthesized from *Trichoderma* sp. displayed excellent antifungal activity against *Candida albicans* which is the major cause of worldwide candidiasis with a minimum inhibitory concentration of 35.5 μg/mL. Another finding in which *Phormidium* sp. derived CuO NPs were observed inhibition against *C. albicans* with maximum inhibition having MIC value of 125 μg/mL (Asif et al., 2023).

Anticancer activity

Cancer is one of the main causes of death worldwide. Cancer has been treated using a variety of techniques including surgery, chemotherapy and radiotherapy. However, these techniques have drawbacks such as cost and additional side effects. For this reason, non-toxic, inexpensive treatments with minimal adverse effects are required. In comparison to other larger biological molecules like enzymes and receptors, nanoparticles have a nanosize (1-100nm). They also have a unique shape, stability and unique interaction with biomolecules that may help diagnose and cure cancer. Several cases have been documented that state the anticancerous property of copper oxide nanoparticles (Waris et el., 2021). Bioinspired copper oxide nanoparticles have the potential to exhibit anticancer properties against several cancer cell lines, in addition to other nanoparticles. Copper oxide nanoparticles target cancer cell lines by a variety of methods, including DNA fragmentation and chromosomal aberration, contact with intracellular macromolecules that trigger cell death and apoptosis, loss of membrane function and cellular leakage as shown in Fig.2.

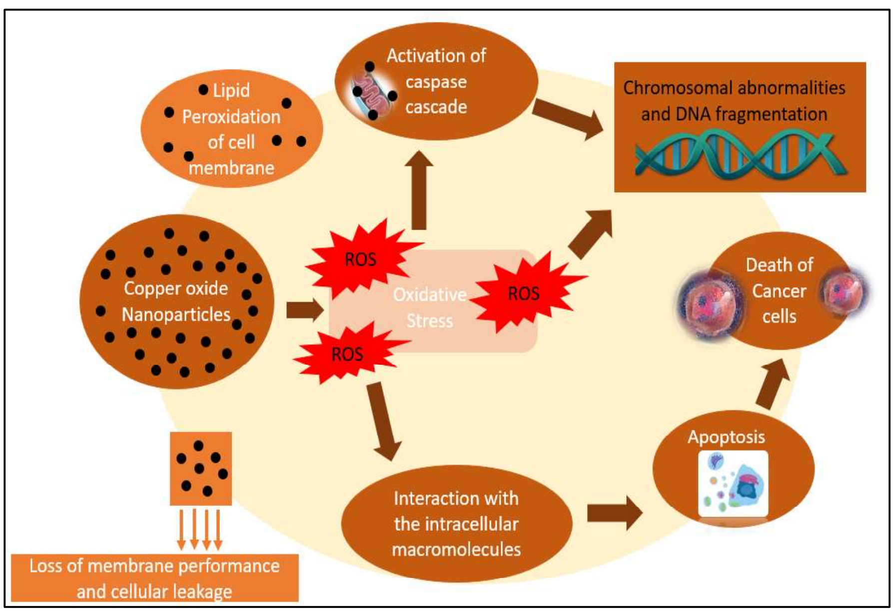

Fig.2: Mechanism of action of Copper nanoparticles against cancerous cells.

The anticancerous activity of copper oxide nanoparticles synthesized from *Annona muricata* plant extract showed strong pro-apoptotic and anti-proliferative effects on breast cancer cell line (AMJ-13 and MCF-7) (Mahmood et al., 2022). Another research work carried out by Tabrez et al. (2022) in which pumpkin seed extract was used to synthesized copper oxide nanoparticles showed 50% inhibition at 25 µg/mL against HCT-116 cell line of Human colorectal cancer. CuO-NPs synthesized using *Abelmoschus esculentus* fruit extract was found to have a considerable anticancer efficacy against the HeLa cancer cell line in 24, 48 and 72 hours (IC_{50} = 1000, 500 and 250 g/mL, respectively). In contrast, the normal fibroblast L929 cell line saw less cytotoxic effects from CuO-NPs than from the cancer cells (Javid-Naderi **et al**., 2023).

Sensors for biomolecule detection

Copper oxide nanoparticles are used for the detection of various biomolecules such as glucose, hydrogen peroxide, dopamine, cholesterol, lactate and as an immunosensor for the Immune complex. A few of them are described below and mentioned in Fig. 3.

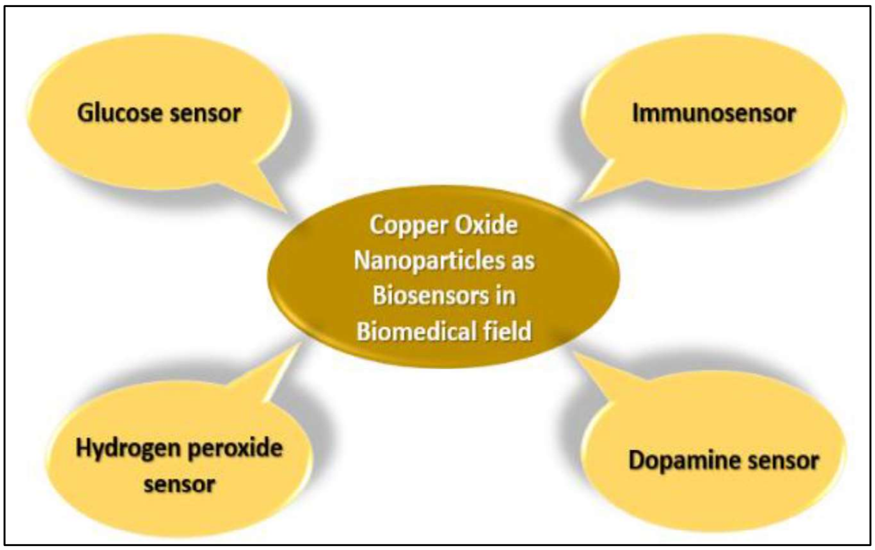

Fig. 3: Copper oxide nanoparticles as a biosensor for the detection of biomolecule.

a) **Glucose sensors:** The World Health Organisation reports states that since 1980, the percentage of people worldwide who have diabetes has nearly risen from 4.7% to 8.5%. Even though the first glucose monitoring sensor was created using an enzyme-based sensor, a nonenzymatic sensor has become more popular recently due to concern about the stability and complexity of the enzymes or other biomolecules. CuO nanoparticles increase the rate of electron transfer on the electrode surface, making them a great platform for glucose electro-oxidation. A non-enzymatic glucose sensor was created using a pencil graphite electrode (PGE) that has been altered by copper oxide nanoparticles and embellished with reduced graphene (Pourbeyram et al., 2019).

b) **Hydrogen peroxide sensors:** Clinical and pharmacological research requires hydrogen peroxide (H_2O_2), an important intermediate in many biological activities. For identifying oxidative stress and hypoxic conditions in cells and tissues, H_2O_2 estimation is essential. CuO nanoparticles are reported to mimic the peroxidase activity and can oxidize a variety of chemical substances. An electrochemical sensor for the detection of hydrogen peroxide (H_2O_2) was developed using cuprous oxide/silver (Cu_2O/Ag) nanocomposites, which were made in a simple one-step method (Yang et al., 2019).

c) **Immunosensors:** Since disease-related protein biomarkers are often found at ultralow levels and hence necessitate ultrasensitive technologies, highly precise and sensitive detection of trace amounts of analytes is a prerequisite for early point of care diagnosis. Regarding this, a promising method based on antigen-antibody interaction in which the immunological components are immobilised directly on the transducer surface is offered by electrochemical immunosensors (EIA). Numerous nanomaterials have been applied to EIA thus far to improve the electrochemical signal and electron transfer capacity, resulting in extremely sensitive sensing. An automated technique for the preparation of samples and signal conversion was used to develop an immunosensor based on CuO NP for Zearalenone detection. CuO nanoparticles have been shown to have a synergistic impact and quicken signal transmission (Xuan et al., 2021).

d) **Dopamine sensors:** Dopamine (DA) is a catecholamine neurotransmitter that is crucial for controlling the activity of the cardiovascular, renal and central neurological systems in mammals. A low level may cause some neurological conditions such as Parkinson's, Alzheimer's, Huntington's schizophrenia, epilepsy and senile dementia. Normally, it is present at a level of 10^{-8}– 10^{-6} M. Using *Artemisia absinthium* leaf extract as a reducing agent and the enzyme tyrosinase (Tyr) as a sensing material, copper oxide nanoparticles (NPs) were synthesized using a green method. P-Cu_2O+ Tyr (PT) together demonstrated a DA-selective response. According to this research, PT can be used as a fluorescent probe for detecting DA (Pavithra et al., 2023).

Conclusion

Over the past decade, there has been a remarkable advancement in the synthesis and biological applications of nanomaterials. Metal oxide nanomaterials research is still in its early stages, with the majority of studies concentrated on metallic nanostructures. CuO nanoparticles can be adjusted at different levels to create innovative and desirable features. They have several interesting physicochemical properties. The potential for developing *in-vitro* and *in-vivo* sensing and therapeutic applications in the biomedical arena is increased by CuO nanoparticles; nevertheless, the combined theranostic use of CuO nanostructure is still unexplored. CuO nanoparticle use is currently paving the way for the identification of biomarkers linked to conditions like diabetes, cancer, stress, hypoxia, neurological illnesses and cardiac syndromes. CuO nanoparticles have great sensing properties, thus more research on using them to create miniature implantable sensors for real-time monitoring should be done. It has become clear that CuO nanoparticles' potency might be increased in a composite form to combine the various functions of each substance. Advanced therapeutic medication substitutes called nanomedicines have the power to revolutionise current perspectives on human health and illness. It results in the emergence of a brand-new area of study known as "nanobiotechnology," an interdisciplinary subject that combines clinical research with nanotechnology on a single foundation to meet the need for more study. The selection of parameters such as particle size, shape, concentration, surface features and operating environment is crucial for the potential biomedical applications of CuO nanoparticles. Neglecting these parameters could limit their broad range of potential uses. To summarise, a better understanding of the phenomena surrounding metal oxide nanoparticles, such as their nucleation process, growth mechanism, biocompatibility, toxicity and surface chemistry, will help regulate the nanomaterial interface and influence how biomolecules interact with it in a variety of biomedical applications.

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