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BIOSYNTHESIS OF COPPER NANOPARTICLES USING *Aristolochia bracteata* LEAF AND EVALUATION OF HEPATOPROTECTIVE ACTIVITY

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ABSTRACT

Nanotechnology is amongst the most widely used technologies in translational research. Nanotechnology deals with particles of a size ranging from 1 to 100 nm. The development of metallic nanoparticles employing biological materials by an eco-friendly approach. Plant mediate nanoparticle can be the best source of such antioxidants and mediate hepatoprotective activity. Nanoparticle based drug delivery can rapidly improves the therapeutic potential of biological active agents. Carbon tetrachloride (CCl_4) is an abundant hepatotoxin used for induction of hepatic cytotoxicity as it is metabolized in the liver releasing free radicals which results in lipid peroxidation and hepatocytes necrosis. The green synthesis NPs drugs is very important especially because it is rarely to find synthetic drugs being used as effective hepatoprotectives. In the aim of present study evaluation of hepatoprotective activity in CuO NPs synthesized using *Aristolochia bracteata* leaves against CCl_4 induced liver cell culture. The present results strongly suggest that rich source of phytochemicals like phenols, flavonoids and terpenoids are confirmation of phytochemical techniques used. *Aristolochia bacteata* leaves extract biomolecule coated CuO NPs formation indication of blue to green colour development and UV-Visible spectrum absorbance occurs at 390 nm confirm of CuO NPs synthesis while CuO NPs size range 42 to 67 nm using SEM. CuO NPs was exposure showed potential *in vitro* hepatoprotective effect against CCl_4 induced liver cell culture and could be used as an effective hepatoprotective nanodrug. *Aristolochia bacteata* leaves natural source can offer advantages to chemical drug and all of which could have positive safe environmental impacts.

Keywords: CuO NPs, *Aristolochia bacteata* leaves, Hepatoprotective.

INTRODUCTION

The most successfully studied nanoparticles today are those made from noble metals, in particular Cu (copper), Ag (Silver), Ti (Titanium), Pt (Platinum), Au (Gold) and Pd (Palladium). Nanoparticles of noble metals, such as copper, gold, silver, and platinum, are widely applied in products that directly come in contact with the human body, such as shampoos, soaps, detergent, shoes, cosmetic products, and toothpaste, besides medical and pharmaceutical applications (Ankanna *et al.*, 2010).

Copper oxide nanoparticles (CuO NPs) have been mainly used as preliminary material due to its natural abundance, low cost production, non-toxic nature with good electrical and optical properties. The extensive use of CuONPs has increased concerns over their potential toxic impacts on ecosystem and human health due to their discharge from

different products to the environment (Chen *et al.* 2012).

Plant leaf extract is mixed with metal precursor solutions to synthesis of nanoparticle at different reaction conditions (Mittal *et al*., 2013). The parameters determining the conditions of the plant leaf extract (such as types of phytochemicals, phytochemical concentration, metal salt concentration, pH, and temperature) are admitted to control the rate of nanoparticle formation as well as their yield and stability (Dwivedi and Gopal, 2010). The phytochemicals present in plant leaf extracts have uncanny potential to reduce metal ions in a much shorter time as compared to fungi and bacteria, which demands the longer incubation time (Jha *et al.,* 2009). Therefore, plant leaf extracts are considered to be an excellent and benign source for metal as well as metal oxide nanoparticle synthesis. Additionally, plant leaf extract play a dual role by acting as both reducing and stabilizing agents in nanoparticles synthesis process to facilitate nanoparticles synthesis (Malik *et al*., 2014).

Flavonoids contain various functional groups, which have an enhanced ability to reduce metal ions. The reactive hydrogen atom is released due to tautomeric transformations in flavonoids by which enol form is converted into the keto form. This process is realized by the reduction of metal ions into metal nanoparticles (Ahmad *et al*., 2010).

Hepatoprotective

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. Liver cell injury caused by various toxicants such as certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes are common. Enhanced lipid peroxidation during metabolism of ethanol may result in development of hepatitis leading to cirrhosis (Agarwal, 2001).

Liver plays a vital role in the elimination of xenobiotics that can induce hepatotoxicity in lever cell culture. CuO nanoparticles have evolved recently as an alternative in various industries and are used for their biomedical applications. *Aristolochia bracteata* plant that has been used in the traditional medicine of South India for its biological active properties. In present study biosynthesis of copper nanoparticles using *Aristolochia bracteata* leaf and evaluation of hepatoprotective activity.

MATERIALS AND METHODS

Collection of sample

The leaves powder of *Aristolochia bracteata* were purchased in March 2022 from siddha medicinal shop, Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation for extract

1 gram of the powder of leaves (*Aristolochia bracteata*) were transferred in to different conical flask (250ml). The conical flask containing 50ml of different solution (ethanol, aqueous and 70% hydroalcohol). The conical flask containing leaves powder were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Synthesis of copper oxide nanoparticles using leaves extract

The copper acetate monohydrate (2.8 g) was dissolved in deionized water (500 ml) and stirred magnetically for 5 min at room temperature. Afterwards, added drop wise *Aristolochia bracteata* leaves aqueous extract under stirring as soon as the leaves extract comes in contact with copper ions to change the colour from blue to green color. After 10 min, the formation of water soluble monodispersed copper oxide nanoparticles were observed (Ghidan *et al*., 2016)

UV-Visible Spectroscopic, FTIR and SEM analysis of CuO NPs

The copper oxide nanoparticles were examined under UV and visible spectrophotometer analysis. The copper oxide nanoparticles were scanned in the wavelength ranging from 380-800 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation. Scanning Electron Microscope (SEM), in this research work, Jeol JSM-6480 LV SEM machine was used to characterize mean particle size and morphology of nanoparticles. The freeze dried sample of CuONPs solution was sonicated with distilled water and small drop of this sample was placed on glass slide and allowed to dry. A

thin layer of platinum was coated to make the samples conductive. Jeol JSM-6480 LV SEM machine was operated at a vacuum of the order of 10-5torr. The accelerating voltage of the microscope was kept in the range 10-20kV.

***In vitro* hepatoprotective activity** (Prakash *et al.*, 2015)

The livers were excised and weighed in a tared beaker of cold calcium-free Locke's solution. Sufficient solution was .removed to give a ratio of 1 g of liver to 10 ml of final suspension. The liver and solution were then transferred to a homogenizer tube, and the liver broken up by pressing down with a loose-fitting lucite pestle. This was followed by twenty even up and-down strokes by hand. Shreds of connective tissue containing many cells remained after this treatment, but they were readily removed by straining through bolting silk. Experience has shown that further homogenization to release more whole cells. The isolated hepatocytes were cultured in Ham's F12 medium, supplemented with 10% newborn calf sample, antibiotics, dexamethasone and bovine insulin. The cell suspension was incubated at 37 ºC for 30 min in a humidified incubator under 5% CO_2. After incubation of 24 hrs, the hepatocytes were exposed to the fresh medium containing CCl_4 (1%) along with different concentration of CuO NPs (100, 200 and 400µg/ml). After 60 min of CCl_4 intoxication, the oxidative markers MDA and liver function SGOT, SGPT were determined.

Experimental Design

Group I: Normal

Group II: Carbon tetrachloride treated alone (1%)

Group III: Carbon tetrachloride + 100µg/ml CuO NPs

Group IV: Carbon tetrachloride + 200 µg/ml CuO NPs

Group V: Carbon tetrachloride + 400 µg/ml CuO NPs

Estimation of oxidative stress and liver function

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Activity of Serum glutamic-oxaloaetic transaminase (SGOT) or Aspartate aminotransferase (AST) and Activity of alanine transaminase (ALT) or SGPT by the method of Reitman and Frankel (1957)

Statistical Analysis

The results were presented as Mean ± SD. Data were analyzed by one-way ANOVA followed by post-hoc Tukey HSD test, data were statistically significant level alpha 0.05 using SPSS ver 20.

RESULTS AND DISCUSSION

In the present study the impact of CuO nanoparticles synthesized using aqueous extract of *Aristolochia bracteata* leaves were investigated on *in vitro* hepatoprotective activity induced with carbon tetrachloride (CCl_4). CCl_4 is known to intoxicate the liver cell culture which can be easily observed by examining the oxidative stress marker and liver profile.

Phytochemical screening

Medicinal plants are important species of plants that according to the traditional medicinal practices and also from modern scientific studies are useful for medicinal purposes to alleviate diseases. These plants are contemplated as rich sources of ingredients that can be used in the synthesis and production of drugs (Oladeji *et al.* 2019). Natural products and their derivatives exhibit minimal side effects and improved efficacy than other synthetic counterparts (Batiha *et al.* 2020). These plant-derived components like flavonoids, quinine, terpenoid, etc conduct certain biological functions that enhance therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and antioxidant properties (Batiha *et al.* 2020).

In the present study was carried out on the *Aristolochia bracteata* leaves extract revealed the presence of medicinally active constituents. The phytochemical characters of *Aristolochia bracteata* leaves presence of tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, polyphenol, glycoside, antroquinone, and coumarins in both aqueous, ethanol and hydroalcoholic extract while alkaloids was absence only in aqueous extract.

The plants containing phenolic compounds could be useful as an antioxidant. Quinine showed antipyretic property so the plants containing quinine like Ocimum, Nyctanthes, Mentha, etc could be used to reduce fever. Mentha is also used as a soothing agent, for relieving toothache, and also as an anti-bacterial anti-helmintic agent (Patil and Godghate, 2016). Nyctanthes, Zingiber also plays a role in maintaining blood sugar. Zingiber, Acorus, Curcuma consisted more amount of more cardiac glycoside which is beneficial for the heart. The phenolic

compound, tannin, terpenoid, flavonoids possess an anti-helmintic property so the plant Zanthoxylum, Acorus could be used to treat stomach problems (Nath and Yadav, 2016). Similarly, *Aristolochia bracteata* leaves was rich source secondary metabolites like phenols and flavonoids, secondary metabolites has a good potential biological active agent and reducing molecule.

Synthesis of Copper oxide Nanoparticles

A study on phytosynthesis of copper oxide nanoparticles by the aqueous leaves extract of *Aristolochia bracteata* leaves was carried out in this work. During the visual observation, copper acetate and bark extract stirred magnetically showed the green mixture after 10 min. The appearance of blue color of copper ions to green color is clear for the development of water soluble monodispersed copper oxide nanoparticles we have demonstrated the efficiency of *Aristolochia bracteata* leaves extract in the rapid synthesis of copper oxide nanoparticles possessing a variety of fascinating morphologies owing to its diverse groups of phytochemicals like phenolics, flavonoids, polyphenols, tannin, anthraquinones, terpenoids and saponin (Plate 1).

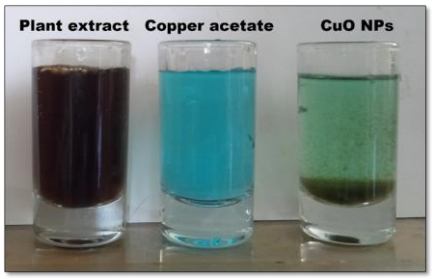

Plate 1: Synthesis of Copper oxide Nanoparticles using *Aristolochia bracteata* leaves

Nanotechnology is a broad interdisciplinary area of research, development and industrial activity which has grown very rapidly all over the world for the past decade. It plays a vital role in technologies of new millennium. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment (Ghorbani *et al*., 2011). Present study agreement with Shiny *et al* (2019) who showed the presence of copper oxide nanoparticles was guajavated by change in colour from bluish green to dark green. In the UV–Vis spectra of the reaction mixture of copper acetate solution with *Aristolochia bracteata* leaves extract the peak was observed at 390nm (Figure 1) guajavating the presence of copper oxide nanoparticles which is synthesized by *Aristolochia bracteata* leaves extract.

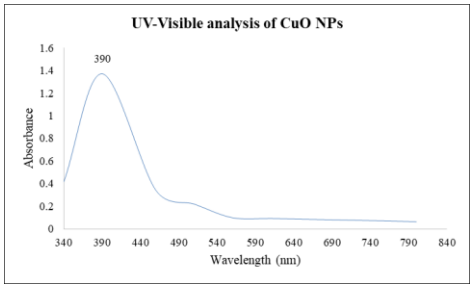

Figure 1: UV–Vis spectra analysis of CuO NPs

Fourier Transform Infra-Red spectral analysis of CuONPs

FTIR spectrum of copper oxide nanoparticles was examined to identify the potential bioactive compounds responsible for capping and efficient stabilization of the CuO nanoparticles synthesized from leaves extract. The peaks observed (Figure 2) for phytochemicals capped CuO nanoparticles formed by reduction by *Aristolochia bracteata* leaves at 3250.47 (Alcohols, Phenols), 1401.53 (Aromatics) and 1181.62, 712.82, 598.85, 562.96 (Alkyl halides) suggest the presence of flavonoids and phenols adsorbed on the surface of copper nanoparticles. These studies suggested that presence of phytochemicals in plant extracts are the key component in reduction and stabilization of copper ions (Thit *et al*., 2013). The similar results has been reported in literature where CuO NPs was synthesized using different leaves extracts (Renu Sankar *et al*., 2014).

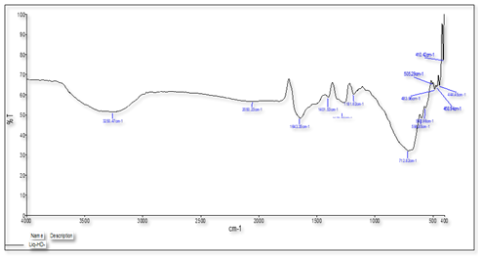

Figure 2: Fourier Transform Infra-Red spectral analysis of CuO NPs

Scanning Electron Microscopical (SEM)

SEM analysis was carried out to understand the topology and the size of the CuONPs, which showed the synthesis of higher density polydispersed spherical CuONPs of various sizes that ranged from 42 to 67 nm respectively as well cubic and crystalline nature of the nanoparticles. Most

of the nanoparticles gathered and only a little of them were dispersed, when observed under SEM in plate 2. Similarly our study close agreed with the previously published results of green synthesized CuONPs (Pugazhendhi *et al.,* 2018). The high agglomeration among the particles was due to electrostatic attraction. It was found that the shape of NPs formation is dependent on the type of biomolecules in the plant extract (Radhakrishnan *et al.,* 2021). In general, it is observed that the biosynthesized CuO NPs showed small and well uniform-sized spherical NPs which is in agreement with the previously reported literature (Andualem *et al.,* 2020). In their research on green chemistry of CuONPs nanoparticles, Suresh *et al.,* have investigated that the copper oxide nanomaterial has spherical shape and is uniformly dispersed (Suresh *et al.,* 2020).

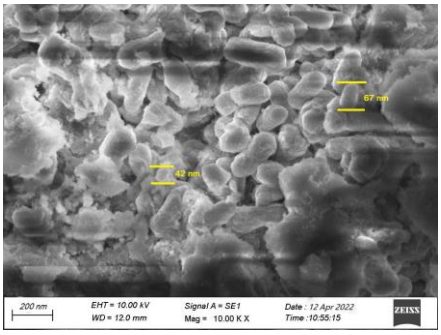

Plate 2: Scanning Electron Microscopical (SEM) analysis of CuONPs

In vitro Hepatoprotective activity of CuO NPs

The liver plays an important role in metabolism and detoxification of compounds which enter the body and may cause hepatic injury, leading to life-threatening diseases. Therefore, major toxicological problems associated with several diseases have been centered around the effects on the liver. Usually, liver cells are affected by hepatotoxic agents through the induction of oxidative damage. Drugs of both synthetic and natural origin are available for treatment of liver diseases. Natural remedies have long been used for treatment of liver diseases. Based on this, protective effects of plant-based herbal medicines against drug-induced toxicity (Singh *et al.,* 2016; Hong *et al.,* 2015).

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome *P*450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($^{\bullet}CCl_3$). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloro methylperoxyl free radical leads to initiate the process of lipid peroxidation., the destruction of Ca^{2+} homeostasis, and finally, results in cell death (Recknagel *et al*., 1989).

These result in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver damage (Recknagel and Glende, 1973; Reckengel *et al*., 1991). MDA is a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions (Ray and Husain, 2002). Hepatotoxic compounds like CCl_4 are known to cause marked elevation in antioxidant status activities and liver function profile. In the present study, treatment with CuO NPs in figure 3 and 4. Hepatoprotective activity of CuO NPs each parameter wereGroup II, Group III and Group IV was statistically significance deference ($P < 0.05$) and Group V was statistically non-significant ($P > 0.05$) compared with Group I.

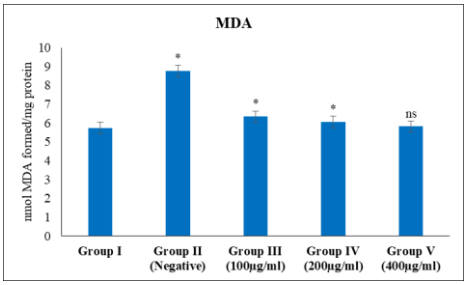

Figure 3: Effect of CuO NPs on MDA activity in experimental group

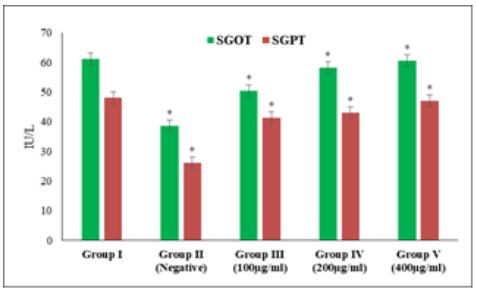

Figure 4: Effect of CuO NPs on Liver profile activity in experimental group

Values were expressed as mean ± SD for triplicate in each group. Data were analyzed by one-way ANOVA followed by

post-hoc Tukey HSD test. Statistically significant variation was derived by comparing Group I versus Group II, Group III, Group IV and Group V. Significance level α 0.05. **P*<0.05, statistically significant and NS= Non significant (*P*>0.05) compared with Group I (Normal).

Necrosis or membrane damage releases the enzymes into circulation; and therefore, they can be measured in hepatocyte. Elevated levels of hepatocyte enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver (Wolf, 1999). The mechanism by which transaminase reaches the circulation is uncertain; leakage from the bile canaliculi into hepatic sinusoids may result from leaky tight junctions and the other hypothesis is that the damaged liver fails to excrete transaminase made in the liver (Thapa and Walia, 2007).

Co-treatment with CuO NPs at doses of 100, 200 and 400µg/ml, significantly prevented the rise in the levels of the marker enzymes, as well as it significantly prevented the decrease in the total protein. Similarly our study agreed with Prakash *et al*, (2015) study on hepatocytes have become valuable tools to evaluate the possible protective effect of drugs in the recent past. The techniques for high yield hepatocytes are made it as useful model. The study was aimed to synthesize the nanoparticles and evaluate the *In vitro* hepatoprotective activity of *Azima tetracantha* leaf extract and Silver nanoparticle (100, 200 and 300µg/ml) through CCl_4 induced toxicity in hepatocytes. In our study conclude CuO NPs synthesis from *Aristolochia bracteata* leaves good hepatoprotective active properties.

CONCLUSION

The present results strongly suggest that rich source of phytochemicals like phenols, flavonoids and terpenoids while *Aristolochia bacteata* leaves extract biomolecule coated CuO nanoparticles exposure showed potential hepatoprotective effect against CCl_4 induced liver cell culture and could be used as an effective hepatoprotective nanodrug. *Aristolochia bacteata* leaves natural source can offer advantages to chemical drug.

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