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RESEARCH ARTICLE

Antioxidant and Anti-Inflammatory Property of Copper Nanoparticles (Cunps) Synthesised using Blue Tea

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ABSTRACT

Introduction: In recent studies copper nanoparticles have drawn attention due to its extensive properties in medicine for curing various diseases and stabilising our immunity. Blue tea, which comes from the Clitoria ternatea plant, also known as the butterfly-blue pea, blue-pea, or Asian pigeonwings, has been around for centuries. It's become increasingly common as a result of its numerous health benefits. This herbal blue tea is really a tisane, which means it's caffeine-free.

Aim: The aim of the study was to evaluate the anti-inflammatory and antioxidant properties of copper nanoparticles synthesised using blue tea.

Materials and Methods: Preparation of the blue tea extract, synthesis of copper nanoparticles, preparation of nanoparticles powder and then antioxidant and anti-inflammatory activity of nanoparticles were analysed. Antioxidant activity and anti-inflammatory activity was assessed using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay and inhibition of albumin denaturation assay. The standard used was diclofenac sodium in various concentrations.

Results: The formation of copper nanoparticles was indicated by the peak found in the spectroscopy. Copper nanoparticles synthesized using blue tea showed highest absorbance at a concentration of 50µl (85.2%) when it was subjected to DPPH assay to check for its antioxidant property at a wavelength of about 517 nm. Copper nanoparticles synthesized using blue tea showed highest absorbance at a concentration of 50µl (91.4%) when it was subjected to inhibition of albumin denaturation assay to analyse for its anti-inflammatory activity at a wavelength of about 660 nm.

Conclusion: The present study suggests that blue tea-mediated copper nanoparticles have good antioxidant and anti-inflammatory properties. It may be concluded that antioxidant and anti-inflammatory activity of blue tea is not as effective as Diclofenac, but it can be more effective when its concentration is increased to acceptable levels.

KEYWORDS:
Antioxidant, Anti inflammatory, Clitoria ternatea, Copper nanoparticles, Green synthesis Innovative.

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INTRODUCTION

The study of an applied science at the atomic or molecular level is known as nanotechnology or nanoscience(1). In recent years, nanoscience and nanotechnology have made huge strides in terms of research and applications. The use of nanotechnology in medicine, specifically drug delivery, is predicted to expand significantly. In pharmaceutical research, nanoparticles are utilised to lower drug toxicity and side effects(2). The fact that these nanoparticles have a far higher surface to mass ratio than other particles, as well as their quantum properties and ability to absorb and transport other

compounds, makes them interesting for medical applications(3).

Nanoparticles have a large (functional) surface on which other substances such as drugs, probes, and proteins can bind, adsorb, and transport. These days, copper, as well as Au, Ag, Pd, and Pt compounds, are widely used(4). Copper is an excellent electrical conductor. As a result of the low prices(5). In today's modern circuitry, this metal is incredibly crucial. Copper nanoparticles have drawn scientists' attention due to its improved catalytic behaviour, electrical conductivity, surface enhanced Raman scattering activity, and greater compatibility, and are predicted to be utilised as critical components in future nano devices(6).

Blue tea, which comes from the Clitoria ternatea plant, also known as the butterfly-blue pea, blue-pea, or Asian pigeonwings, has been around for centuries(7). It's become increasingly common as a result of its numerous health benefits. This herbal blue tea is really a tisane, which means it's caffeine-free(8). This rich, bright-color blue, floral-scented blue tea is made by infusing butterfly blue pea flowers in hot water. So it's a Tisane, which is a herbal concoction produced by infusing leaves or herbs. The butterfly pea plant is a member of the Fabaceae pea family and is native to South Asia(9). Blue tea has been shown to have a variety of health benefits, including immune boosting properties, enhanced cognitive capacity, and weight loss in recent studies(10). Blue tea has been used as a memory enhancer, nootropic, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilization, and sedative agent for decades, according to a study published in the Journal of Ethnopharmacology(11).

In recent years, nanotechnology research has shifted away from traditional applications and toward green technologies(12)(13)(14)(15)(16)(17). This approach can use natural plant extracts, essential oils, fungus, or bacteria to make nan-oparticles(18–23). Green synthesis aims to blueuce the use and production of hazardous chemicals such as formaldehyde and sodium borohydride, which are commonly used as bleaching agents in traditional procedures(6). Plant extracts are also used as excellent nanoparticle suspension stabilisers in ecologically friendly nanoparticle manufacturing methods, eliminating the need for additional chemical components(24). Our team has extensive knowledge and research experience that has translated into high quality publications(25–44). The aim of the study was to assess the antioxidant and anti-inflammatory properties of copper nanoparticles synthesised using blue tea.

MATERIALS AND METHODS

Preparation of the Extract

In a beaker 1g of blue tea was added to 100ml of distilled water and mixed well. And then boiled for 15 minutes at 70-80°C. The solution was filtered by using Whatman no. 1 filter paper and funnel. The filtered blue extract was collected and stored for further use (Figure 1, Figure 2).

Fig.1: Mixture of blue tea in distilled water.

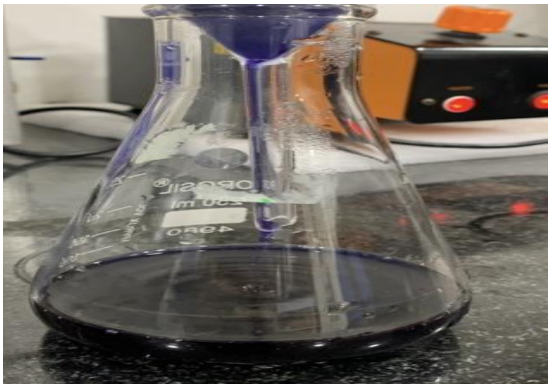

Fig.2: Concentrated extract of blue tea.

Synthesis of Copper Nanoparticles

20mM of CuSO4 was prepablue using distilled water. The blue tea extract was then added, and distilled water was used to make a 100ml solution. Then, using a magnetic stirrer in an orbital shaker, thoroughly mix everything together. To validate the presence of copper nanoparticles produced, the absorbance was measured using a UV - Visible Spectrometer at regular intervals. Every two hours, a reading was taken to observe the colour change. To collect the nanoparticles, the final reaction mixture was centrifuged for 10 minutes. The finished reaction mixture was centrifuged for 10 minutes at 800 rpm in a Lark refrigerated centrifuge. After that, the samples were collected and stoblue in an airtight Eppendorf tube (Figure 3).

Fig.3: Mixture of CuSO4 and blue tea extract.

Antioxidant activity of copper nanoparticles synthesized using blue tea

A test tube rack was arranged with five test tubes, each marked with a label signifying the various concentrations of the extract from 10 to 50 µl. Each test tube was loaded with DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), ethanol and the extract. The DPPH free radical method produces a violet solution in ethanol and is an antioxidant assay based on electron transfer. In the presence of an antioxidant molecule, this free radical, which is stable at room temperature, was diminished, resulting in a colourless ethanol solution. A UV-Spectrophotometer was used to determine the rate of its activity.

Anti inflammatory activity of copper nanoparticles synthesized using blue tea (Inhibition of albumin denaturation assay)

The reagent for the assay was BSA (Bovine serum albumin). BSA (bovine serum albumin) accounts for over 60% of all proteins in animal serum. It's often employed in cell culture, especially when protein supplementation was required but the other serum components were undesirable. When BSA was heated, it denatures and begins to express antigens. 5 test tubes containing varied concentrations (10-50 µl) of blue tea extract were mixed with 2ml of 1 percent Bovine albumin fraction, and the pH of the reaction mixture was adjusted to 6.8 using 1N HCL. In a water bath, the reaction mixture was incubated at room temperature for 20 minutes. The mixture was allowed to cool to room temperature before measuring the absorbance at 660 nm. The standard used was diclofenac sodium in various concentrations.

Formula used for calculating the % Inhibition:

% Inhibition = Control O.D – Sample O.D / Control O.D

RESULTS

Antioxidant activity of copper nanoparticles synthesized using blue tea

UV-Vis Spectroscopy was used to confirm the absorbance of free radicals by the extract subjected to the DPPH assay to analyze its antioxidant activity. The UV – Vis Spectra was recorded for the prepablue copper nanoparticles synthesized using blue tea. From the spectra, it was observed that the extract at 517 nm had the highest absorbance at a concentration of 50 µl (85.2%), indicating significant antioxidant properties, as potent as DPPH itself. This confirms the potent efficacious antioxidant activity of the blue tea extract (Figure 4, Figure 5).

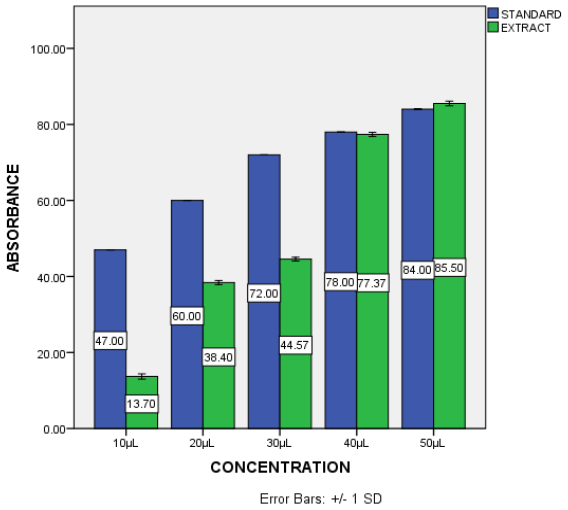

Fig.4: The bar graph shows the comparison of the mean absorbance of antioxidant activity of both standard and blue tea extract at various concentrations. The X axis represents the various concentrations of standard and blue tea extract in units of µL and the Y axis represents the mean absorbance. Blue represents the standard and green represents blue tea. The graph shows that the mean absorbance of blue tea was significantly lesser when compared to the standard at all the concentrations even though the magnitude of difference was lesser at higher concentrations ($p<0.05$) (unpaired t test).

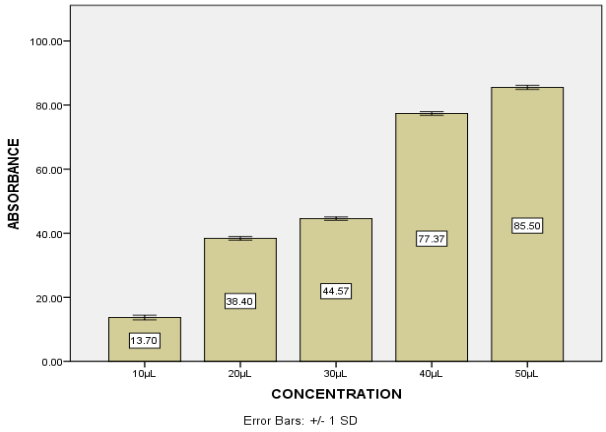

Fig.5: The figure shows the mean absorbance of antioxidant properties of blue tea extract at different concentrations. The X axis represents the various concentrations of blue tea extract in units of µL and the Y axis represents the mean absorbance. There was a significant increase in the mean absorbance from lower concentration to higher concentrations. ($p<0.05$) (One Way ANOVA followed by Tukey's post hoc analysis).

Anti inflammatory activity of copper nanoparticles synthesized using blue tea

UV-Vis spectroscopy is used to confirm the absorbance of free radicals by the extract subjected to the inhibition of albumin denaturation assay to analyze its anti-inflammatory activity. [31] The UV-Vis Spectra was recorded for the prepablue copper nanoparticles using blue tea extract. It was observed from the spectra that the extract at 660 nm had the highest absorbance at a concentration of 50µl (92.2%), which was indicative for significant anti-inflammatory activity, as potent as diclofenac sodium itself. This confirms the potent efficacious anti-inflammatory activity of the copper nanoparticles synthesized using blue tea extract (Figure 6, Figure 7).

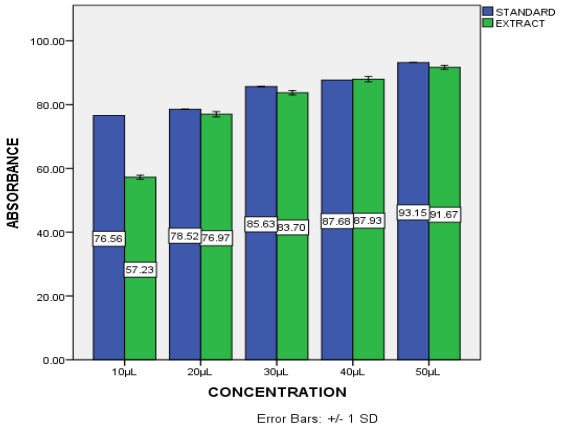

Fig.6: The bar graph shows the comparison of the mean absorbance of anti-inflammatory activity of both standard and blue tea extract at various concentrations. The X axis represents the various concentrations of standard and blue tea extract in units of µL and the Y axis represents the mean absorbance. Blue represents the standard and green represents blue tea. The graph shows that the mean absorbance of blue tea was significantly lesser when compared to the standard at all the concentrations even though the magnitude of difference was lesser at higher concentrations ($p<0.05$) (unpaired t test).

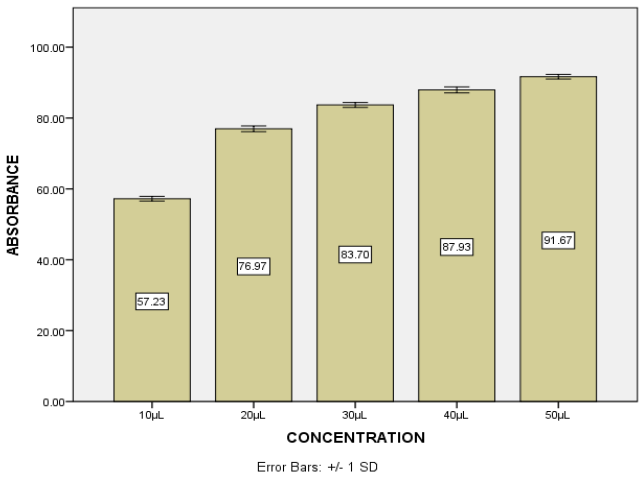

Fig.7: The figure shows the mean absorbance of anti-inflammatory properties of blue tea extract at different concentrations. The X axis represents the various concentrations of blue tea extract in units of µL and the Y axis represents the mean absorbance. There was a significant increase in the mean absorbance from lower concentration to higher concentrations. ($p<0.05$) (One Way ANOVA followed by Tukey's post hoc test analysis).

DISCUSSION

This research was undertaken to assess the anti-inflammatory and antioxidant properties of copper nanoparticles made from blue tea.

In our present study, when subjected to a DPPH assay, blue tea mediated copper nanoparticles displayed the highest absorbance at a concentration of 50µl (85.2%) to check for their antioxidant property at a wavelength of around 517 nm in the current study. Copper nanoparticles produced with blue tea demonstrated the maximum absorbance at a concentration of 50µl (91.4%) when tested for anti-inflammatory action at a wavelength of about 660 nm when submitted to an inhibition of albumin denaturation assay.

The antioxidant and anti-inflammatory activity of Adhatoda Vasica medicated copper nanoparticles was examined by Thariny E et al., and suggested that copper nanoparticles mediated by A. vasica have antioxidant and anti-inflammatory properties. Copper nanoparticles (45) have a powerful catalytic activity that can be employed to alleviate inflammation. There was an other study looked at the antioxidant activity of zinc oxide nanoparticles generated using grape seed extract was examined by Akshaya K et al., who found that the extract at 517 nm exhibited the maximum radical scavenging activity at the concentration of 25L, indicating considerable antioxidant activity(12). Similarly Pranati T et al., investigated the anti-inflammatory and cytotoxic effects of a clove and cinnamon herbal formulation, discovering that the resulting clove and cinnamon extract was a potent antioxidant and antibacterial agent(22).

Another study done by Ethel Jeyaseela Jeyaraj et al., regarding the extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals. The use of maceration or ultrasonic aided extraction considerably improved the production of phytochemicals from C. ternatea flowers (16–247% of increase). C. ternatea flowers have been used to separate phytochemicals such as kaempferol, quercetin, and myricetin glycosides, as well as anthocyanins. Clitoria ternatea flower extracts have been discoveblue to have antibacterial, antioxidant, anti-inflammatory, cytotoxic, and antidiabetic properties, all of which are advantageous to human health(46). Devi BV et al., evaluated the anti-inflammatory efficacy of zinc oxide nanoparticles produced using grape seed extract and found that they are a powerful anti-inflammatory medication with few side effects(47).

In the present study, we tested the antioxidant and anti-inflammatory activities of copper nanoparticles produced with blue tea in a similar way to these investigations. Antioxidants have the potential to bind to free radicals and neutralise them before they cause harm. Some antioxidants are called polyphenols because they have a phenolic ring in their chemical composition. According to laboratory studies, the antioxidant properties of blue tea may aid to prevent DNA damage and inflammation(48) (49) (50) (51) (52) (53) (54) (55) (56) (57) (58) ((58,59) (60) (61). As a result, blue tea has anti-inflammatory properties due to its high flavonoid content. Blue tea possesses antioxidant action, according to the studies described above, but there have been no experiments incorporating blue tea into nanoparticles. Even though the present study suggests that blue tea nanoparticles have antioxidant and anti-inflammatory properties, more clinical trials are needed to confirm these findings for clinical application.

CONCLUSION

The present study suggests that blue tea-mediated copper nanoparticles have good antioxidant and anti-inflammatory properties. It may be concluded that antioxidant and anti-inflammatory activity of blue tea is not as effective as Diclofenac, but it can be more effective when its concentration is increased to acceptable levels.

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CONFLICT OF INTEREST

None declared

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