ANTIOXIDANT AND ANTI INFLAMMATORY ACTIVITY OF COPPER NANOPARTICLES SYNTHESIZED USING RED TEA

Running Title: Antioxidant and anti-inflammatory activity of red tea mediated copper nanoparticles

ABSTRACT:

Background: Recently, nanoparticles have been playing a pivotal role in modern material development. Nanotechnology has provided great possibilities in various fields of science and technology. The antioxidant characteristics of Aspalathus linearis, also known as rooibos, include antimutagenic, anticarcinogenic, anti-inflammatory, and antiviral capabilities.

Aim: To determine the anti-inflammatory and antioxidant activity of copper nanoparticles synthesized using red tea.

Materials and Methods: Preparation of the red 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 red tea showed highest absorbance at a concentration of 50µl (82%) when it was subjected to DPPH assay to check for its antioxidant property at a wavelength of about 517 nm. Copper nanoparticles synthesized using red tea showed highest absorbance at a concentration of 50µl (92.2%) 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 red tea mediated copper nanoparticles showed good antioxidant activity and anti-inflammatory activity. It can be concluded that the antioxidant activity of red tea is not as efficient as Diclofenac but it can be more efficient when it's concentration is raised to safe levels and anti-inflammatory activity of red tea is as efficient as Diclofenac.

Keywords: Copper nanoparticles, Red tea, Antioxidant, Anti-inflammatory, Green synthesis, Innovative.

1. INTRODUCTION:

Nanotechnology, often known as nanoscience, is the study of an applied science at the atomic or molecular level. Nanoscience and nanotechnology have seen tremendous progress in research and applications in recent years. Nanotechnology's application in medicine, specifically drug delivery, is expected to grow substantially(1). Nanoparticles in pharmaceutical research are used to reduce drug toxicity and adverse effects. The fact that these nanoparticles have a surface to mass ratio that is significantly higher than that of other particles, as well as their quantum characteristics and capacity to absorb and convey other substances, makes them appealing for medicinal applications(2).

Nanoparticles have a huge (functional) surface that can bind, adsorb, and transport other substances like medicines, probes, and proteins. Copper, as well as Au, Ag, Pd, and Pt compounds, are commonly employed these days. Copper is a good conductor of electricity(3) (4). Because of the low costs. This metal is extremely important in current electronic circuits. Copper nanoparticles have attracted the attention of scientists for their superior catalytic behaviour, conductivity of electricity, and surface enhanced Raman scattering activity and better compatibility, and are expected to be used in future nano devices as crucial components(5) (6). Nanowires made of copper can be used to make magnetic devices, nanosensors, electron emitters, and other electrical devices and are employed in nanoelectronics(7).

The focus of nanotechnology research has switched significantly from traditional applications to green processes in recent years.(8–14) For the synthesis of nan-oparticles, this method can use natural plant extracts, essential oils, fungus, or bacteria. Green synthesis aims to reduce the use and production of harmful compounds like formaldehyde and sodium borohydride, which are often employed as reducing agents in traditional procedures(15). Plant extracts are also employed as effective stabilising agents of nanoparticle suspension in environmentally friendly methods, which eliminates the usage of additional chemical compounds during nanoparticle synthesis(16). Our team has extensive knowledge and research experience that has translated into high quality publications.(17–29),(30–34) (35) (36). The aim of this study was to determine the anti-inflammatory and antioxidant activity of copper nanoparticles synthesized using red tea.

2. MATERIALS AND METHODS:

2.1. Preparation of the extract:

In a beaker 1g of red 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 extract was collected and stored for further use (Figure 1, Figure 2).

Figure 1: Mixture of red tea in distilled water

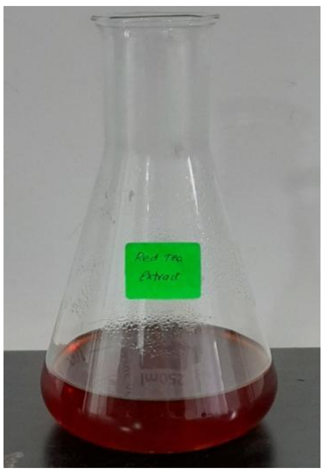

Figure 2: Concentrated extract of red tea

2.2. Synthesis of ZnO nanoparticles:

20mM of CuSO4 was prepared using distilled water. The red 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 stored in an airtight Eppendorf tube (Figure 3, Figure 4).

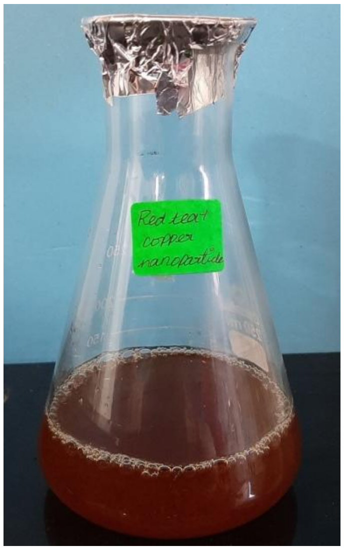

Figure 3: Mixture of $CuSO_4$ and red tea extract

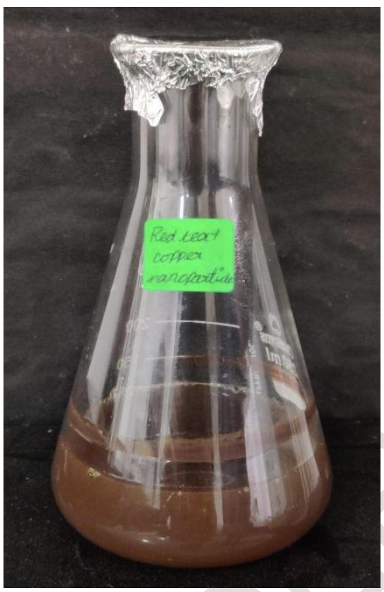

Figure 4: The final reaction solution with red tea mediated copper nanoparticles.

2.3. Antioxidant activity of copper nanoparticles synthesized using red 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.

2.4. Anti inflammatory activity of copper nanoparticles synthesized using red 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 red 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

3. RESULTS

3.1. Antioxidant activity of copper nanoparticles synthesized using red 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 prepared copper nanoparticles synthesized using red tea. From the spectra, it was observed that the extract at 517 nm had the highest absorbance at a concentration of 50 µl (82%), indicating significant antioxidant properties, as potent as DPPH itself. This confirms the potent efficacious antioxidant activity of the red tea extract (Figure 5, Figure 6).

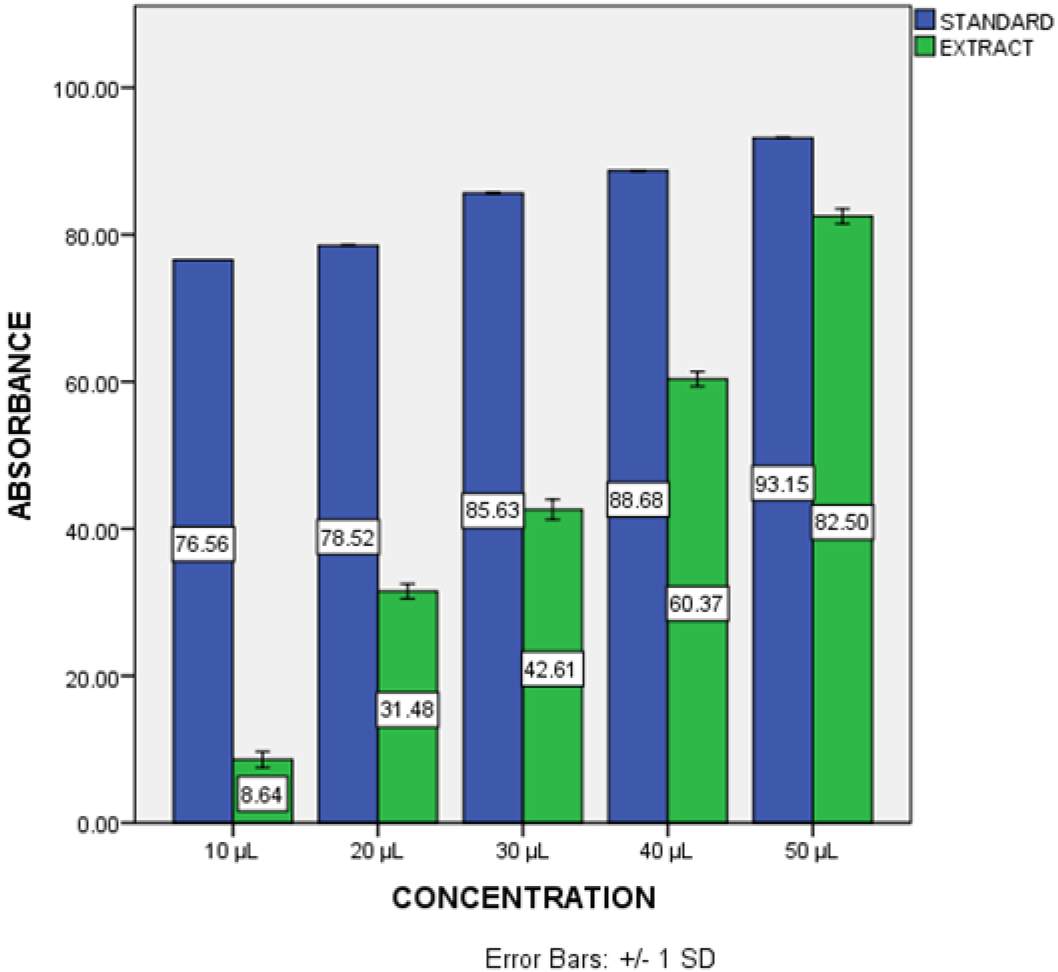

Figure 5: The bar graph shows the comparison of the mean absorbance of antioxidant activity of both standard and red tea extract at various concentrations. The X axis represents the various concentrations of standard and red tea extract in units of µL and the Y axis represents the mean absorbance. Blue represents the standard and green represents red tea. The graph shows that the mean absorbance of red 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).

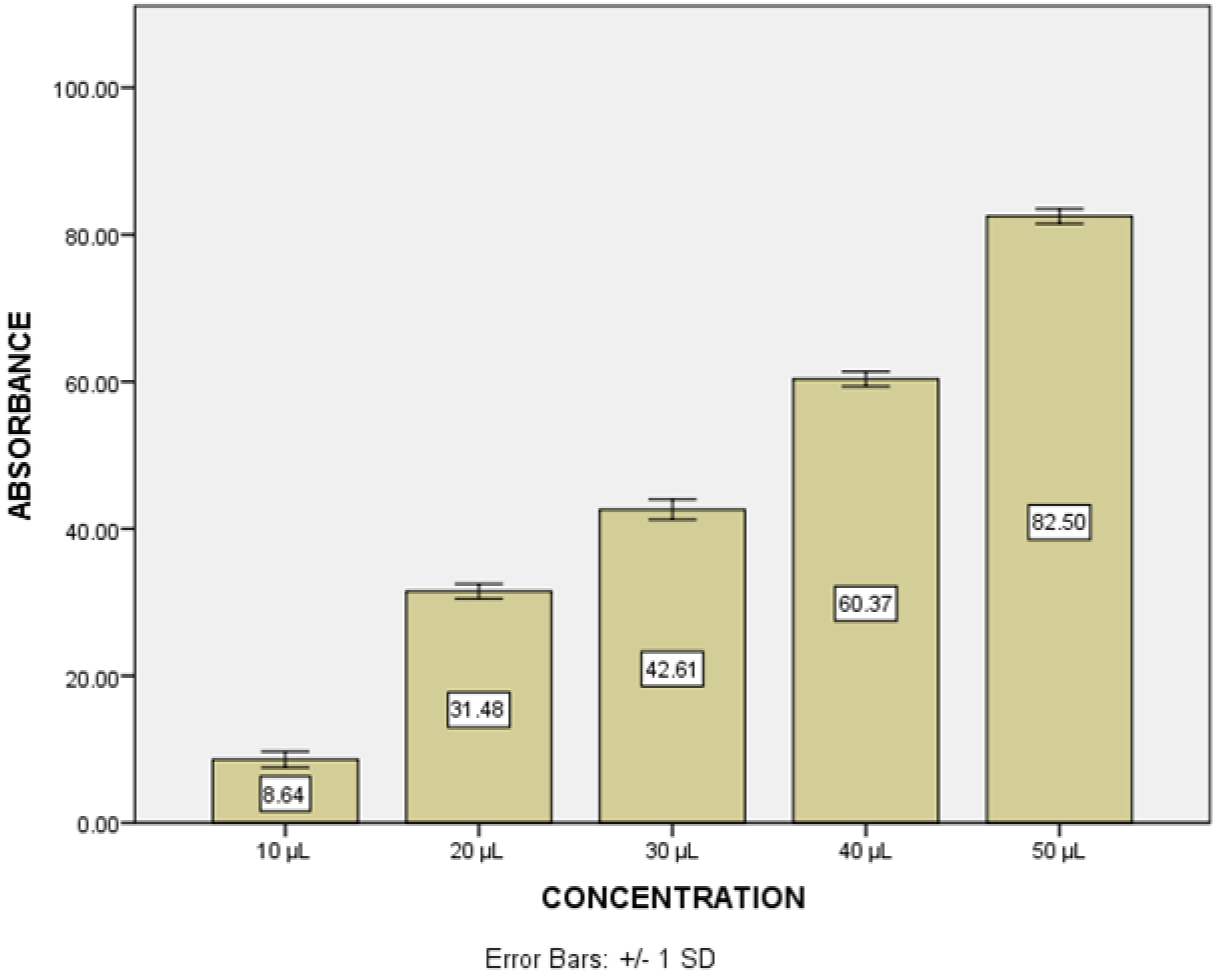

Figure 6: The figure shows the mean absorbance of antioxidant properties of red tea extract at different concentrations. The X axis represents the various concentrations of red tea extract in units of µL and the Y axis represents the mean absorbance. There was a significant increase in the mean absorbance from lower concentration to higher concentrations. ($p<0.05$) (One Way ANOVA followed by Tukey's post hoc analysis).

3.2. Anti inflammatory activity of copper nanoparticles synthesized using red 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 prepared copper nanoparticles using red 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 red tea extract (Figure 7, Figure 8).

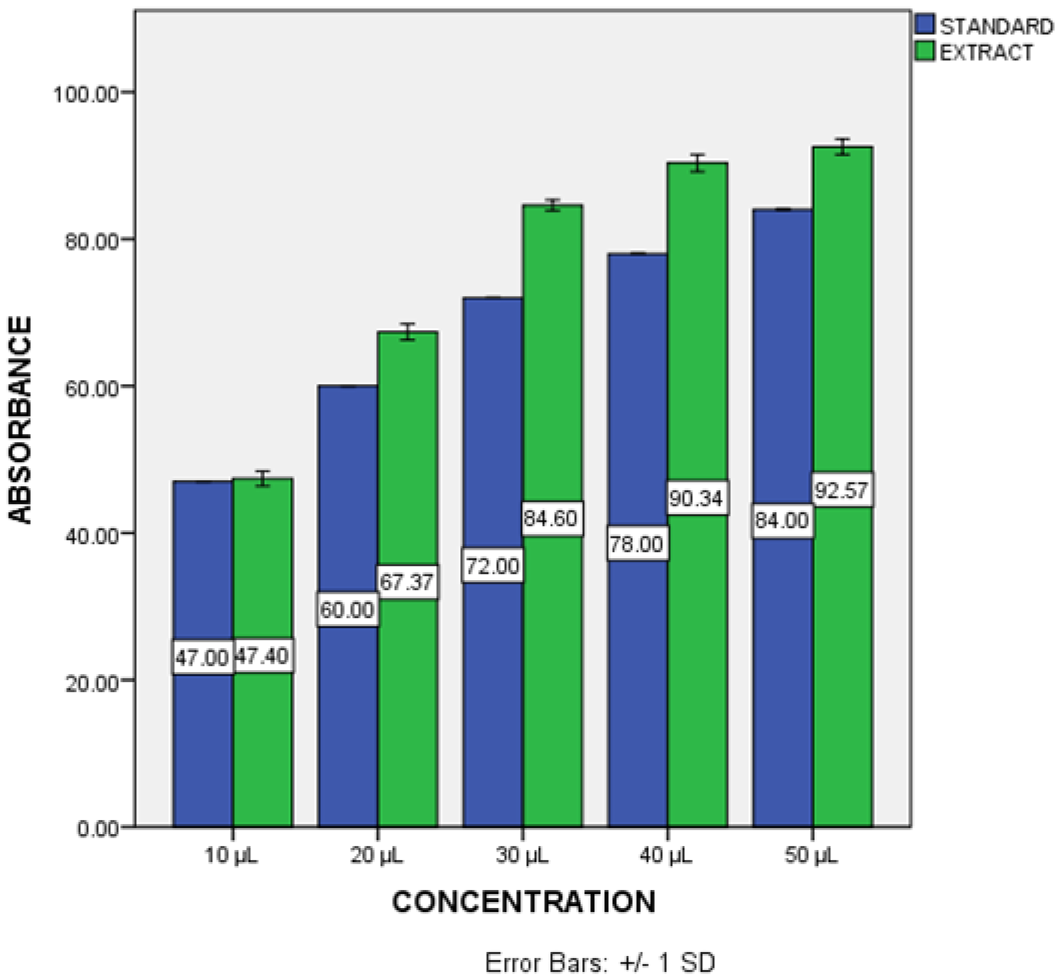

Figure 7: The bar graph shows the comparison of the mean absorbance of anti-inflammatory activity of both standard and red tea extract at various concentrations. The X axis represents the various concentrations of standard and red tea extract in units of µL and the Y axis represents the mean absorbance. Blue represents the standard and green represents red tea. The graph shows that the mean absorbance of red tea was significantly higher when compared to the standard at all the concentrations ($p<0.05$) (unpaired t test).

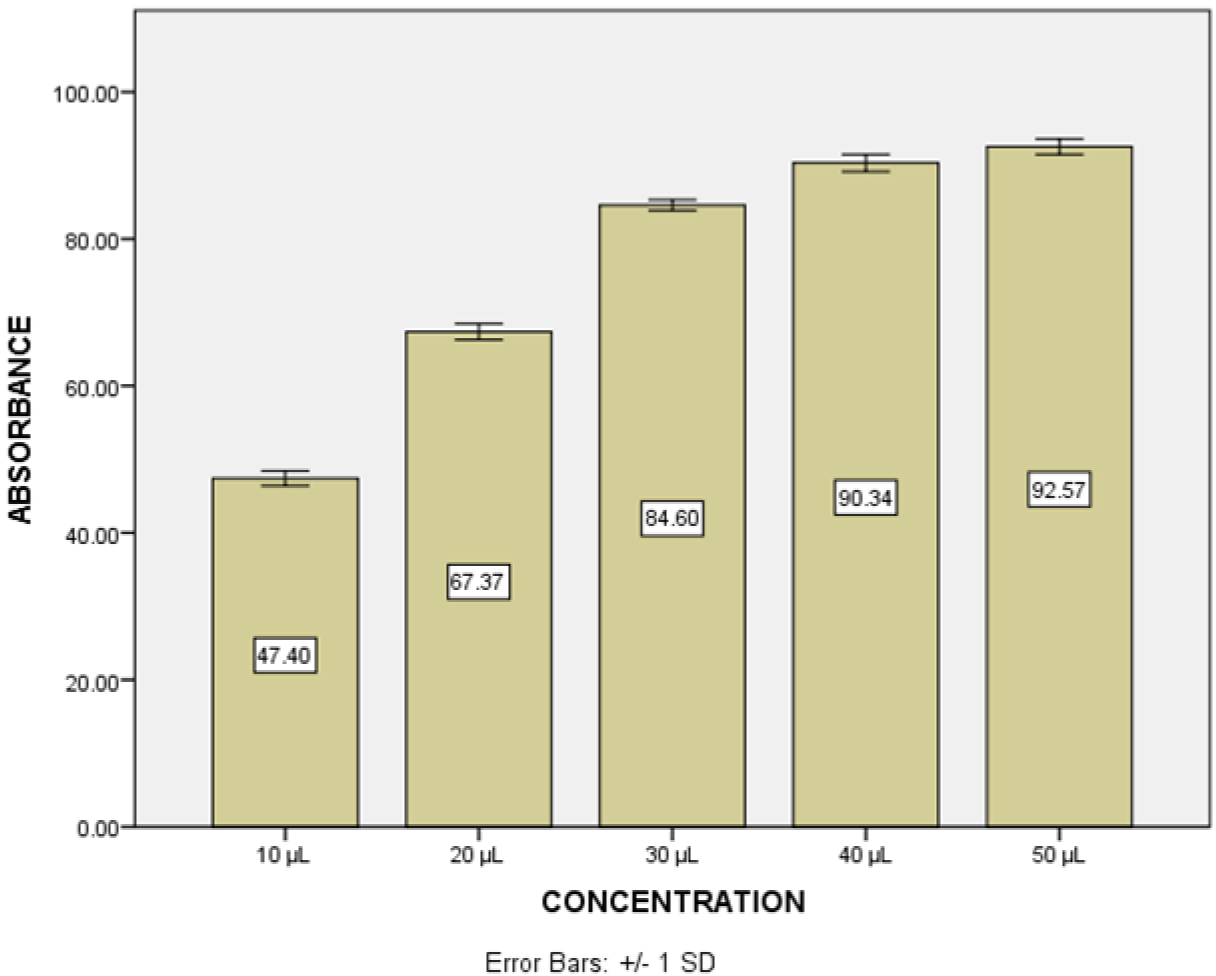

Figure 8: The figure shows the mean absorbance of anti-inflammatory properties of red tea at different concentrations. The X axis represents the various concentrations of red tea extract in units of µL and the Y axis represents the mean absorbance. There was a significant increase in the mean absorbance from lower concentration to higher concentrations. ($p<0.05$) (One Way ANOVA followed by Tukey's post hoc test analysis).

4. DISCUSSION:

This study was done to analyse the anti-inflammatory and antioxidant activity of copper nanoparticles synthesized using red tea.

In the present study, when subjected to DPPH assay it was found that red tea mediated copper nanoparticles showed highest absorbance at a concentration of 50µl (82%) to check for its antioxidant property at a wavelength of about 517 nm. When subjected to inhibition of albumin denaturation assay, copper nanoparticles synthesized using red tea showed highest absorbance at a concentration of 50µl (92.2%) to check for its anti-inflammatory activity at a wavelength of about 660 nm.

The antioxidant activity of zinc oxide nanoparticles generated using grape seed extract was examined by Akshaya K et al, who discovered that the extract at 517 nm exhibited the maximum radical scavenging activity at a concentration of 25 L, indicating considerable antioxidant activity(37). Another study on the anti-inflammatory activity of titanium dioxide nanoparticles synthesised with grape seed extract found that the titanium dioxide nanoparticles synthesised with grape seed extract have good anti-inflammatory activity and could be used as pastes, gels, and other topical applications in the future(38). Another study looked at the anti-inflammatory activity of selenium nanoparticles made with clove and cinnamon. It found that the selenium nanoparticles made with clove and cinnamon have good anti-inflammatory action, which helps to reduce the negative effects (39). Another investigation on the antibacterial and antioxidant activities of clove and cinnamon herbal formulations discovered that the clove and cinnamon extract has antimicrobial and antioxidant potential (40).

Suresh M et al. investigated the antioxidant activity of a Syzygium aromaticum and Cinnamomum verum formulation mediated by silver nanoparticles and discovered that the formulation has an antioxidant impact that rises with concentration (41). The antioxidant activity of grape seed mediated TiO2 nanoparticles was investigated in another work, which revealed efficient and powerful antioxidant activities of grape seed mediated TiO2 nanoparticles(42).

The anti-inflammatory and cytotoxic effects of a clove and cinnamon herbal formulation were studied by Pranati T et al, who discovered that the produced clove and cinnamon extract was a powerful antioxidant and antibacterial agent (13). A prior investigation on the antioxidant activity of clove and cinnamon herbal formulations revealed that clove and cinnamon have the potential to be efficient antioxidants(43). Devi BV et al investigated the anti-inflammatory efficacy of zinc oxide nanoparticles generated using grape seed extract and discovered that the zinc oxide nanoparticles made with grape seed extract are a strong anti-inflammatory drug with few adverse effects(44). The antibacterial, antioxidant, and cytotoxic effects of an aqua alcoholic extract of grape seed were studied by Kandhan TS et al., who discovered the extract's significant antioxidant and antimicrobial activities(45).

Similar to these studies, we have assessed the antioxidant and anti-inflammatory activity of copper nanoparticles synthesized using red tea. Antioxidants have the ability to attach to free radicals before they do harm. Because some antioxidants have a phenolic ring in their chemical structure, they are referred to as polyphenols. Rooibos tea includes polyphenol antioxidants, such as flavonoids and phenolic acids, which are powerful free radical scavengers, according to laboratory studies(46). According to Bramati L et al, Unfermented rooibos had a total antioxidant activity that was 2-fold higher than fermented rooibos. As a result, red tea possesses antioxidant properties (47). Red tea's antioxidant action may help to reduce DNA damage and inflammation. Therefore showing that red tea has anti-inflammatory activity with abundant flavonoids(48). From the above studies mentioned, red tea has antioxidant activity but there are no studies which incorporated red tea in nanoparticles. So, we have analysed the antioxidant and anti-inflammatory activity of red tea. Even though we have found out that red tea nanoparticles have antioxidant and anti-inflammatory activity, these findings need to be confirmed with more clinical trials for clinical application.

5. CONCLUSION:

The present study suggests that red tea mediated copper nanoparticles showed good antioxidant activity and anti-inflammatory activity(49) (50) (51) (52) (53) (54) (55) (56) (57) (58) (59) ((59,60) (61) (62). It can be concluded that the antioxidant activity of red tea is not as efficient as Diclofenac but it can be more efficient when it's concentration is raised to safe levels and anti-inflammatory activity of red tea is as efficient as Diclofenac.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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