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Evaluation of Anti Inflammatory and Cytotoxic Effect of Copper Nanoparticles Synthesised Using Seed Extract of *Mucuna pruriens*

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Authors' contributions

This work was carried out in collaboration among all authors. Collection of literature and drafting of manuscript was done by author PA and revising and the final approval of manuscript was done by authors RVG and SRK. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Nanotechnology is a rapidly developing interdisciplinary area that has brought enormous changes in dentistry. Copper nanoparticle made from plant extract would be an environmental friendly, convenient and dependable way for providing therapeutic agents that are safe, free of side effects and useful for a wide range of diseases. Mucuna pruriens seed extract was selected for our study, due to its due to its anti- bacterial, anti-diabetic, anti- Parkinson, anti-cholesterol and anti- oxidant properties.

Aim: To evaluate the anti-inflammatory properties of *Mucuna pruriens*, the green synthesis, characterization of CuNPs, and screening of their cytotoxic activity.

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Materials and methods: The collection and preparation of *Mucuna pruriens* extract was done and stored for further use. Synthesis of Cu nanoparticles was done with 30 milli molar of copper (II) sulfate. Centrifugation was done and characterisation of Copper nanoparticles using ultraviolet (UV)-visual spectrophotometer. Cytotoxic effect and anti-inflammatory activity of copper nanoparticles with Mucuna pruriens seed extract were assessed using Brine Shrimp Assay at 5µL, 10μ L, 20μ L, 40μ L and 80μ L and Bovine Serum Albumin (BSA) at 5 µL, 10μ L, 20μ L, 30μ L, 50μ L. **Results:** The anti-inflammatory activity of Copper Nanoparticles with *Mucuna pruriens*, increased with increase in concentrations. Percentage of inhibition was 17% at 10 µL concentration, 24% at 20 µL, 43% at 30μ L and 54% at 40μ L and highest at 50μ L (71%). Cytotoxicity of Copper Nanoparticles with *Mucuna pruriens*, at 5 µL concentration there was a death of 10% of nauplii, at 10 µL there was a death of 20% of nauplii, at 20 µL and 40μ L there was a death of 30% of nauplii and at 80μ L there was a death of 40% of nauplii. As the concentration increased, the cytotoxicity of the nanoparticles increased.

Conclusion: Based on the results of the current study, it is concluded that *Mucuna pruriens* mediated Cu Nps can be used as a potential source of anti inflammatory agent and also as an anti cancer drug for the treatment of tumours and cancers.

Keywords: Mucuna pruriens; Copper Nanoparticles; anti inflammatory activity; cytotoxic effect; bovine serum albumin (bsa) and brine shrimp lethality assay (bsla); innovative technique.

1. INTRODUCTION

Nanotechnology is an advanced branch of science that has the potential to solve a wide range of problems in a variety of fields[1]. differentiated Nanoparticles are from conventional materials by their scale, form, distribution and surface-to-volume ratio of nanoparticles[2]. Nanoparticles for metal oxides have received a lot of interest as major applications photovoltaics, nanoscale in electronics. nanoscale sensors, nanoscale devices, information storage and stimulation[3,4]. In the biomedical field, these nanoparticles have been investigated for antimicrobial applications, heavy metal ion sensing, imaging and drug delivery and while for environmental applications, nanoparticles are used for bioremediation of diverse contaminants, water treatment, removal of pollutant dye and production of clean energy. Plant extracts, as a promising approach for nanoparticle synthesis, escape the drawbacks of chemical approaches [5]. Nanoparticles have a wide variety of uses in medicine. As a consequence, one of the most challenging nanoscale researches in recent years has been the synthesis of green chemistry and nanotechnology [6]. Hence, these studies can be useful in the field of nanomedicine for the upcoming generation due to their low cost and extra revenue[7]. Nanoparticles circulate through the body, but they also penetrate cells and have the capacity to bind to specific cells.[8]. Cu nanoparticles is an essential trace element in living organisms, which plays an important role in protein function. These nanoparticles (CuNPs)

have particularly shown high toxicity against tumor cells such as pulmonary adenocarcinoma (A549) and human leukemia monocytic cell lines (THP-1)[9][10]. Copper nanoparticles have a lot of applications, including antibiotic, antimicrobial, and antifungal properties when applied to fibres, coatings, and textiles; high strength metals and alloys and effective catalyst for chemical methanol reactions and and alvcol synthesis[11,12]. Copper nanoparticles have been shown in several experiments to trigger cytotoxicity, genotoxicity, inflammation, and oxidative stress.[13][14]. Cytotoxicity in Cu Nps plays a major role against cancer cell lines. The consistency of being harmful to cells is known as cytotoxicity. The integrity of cell membranes is often impaired by cytotoxic compounds[15]. The main purpose of cancer chemotherapy is to kill cancer cells directly while avoiding toxicity of healthy cells. This is the limitation to the use of several chemotherapeutic agents[16]. It is important to monitor and ensure that these chemotherapeutic drugs are potent and effective prior to patient administration[17]. Hence, selective toxicity must be put in consideration in the discovery of leads for cancer treatment.

Inflammation is the body's main reaction to an illness or injury, and it is vital to our immunity. It arises as a result of vascular tissues' biological response to adverse factors such as bacteria, defective cells, or irritants[18]. Because of the increasing prevalence of pain and pain-related disorders, as well as other health treatment complexities, it is vital to be aware of the antiinflammatory narcotics available on the market[19]. Furthermore, nonsteroidal antiinflammatory drug use would be linked to stomach ulcers, dizziness, headaches, allergic reactions, and heartburn[20]. As a result, it is beyond time for us to return to nature and encourage herbal medicines over allopathic medicines because herbal medicines have less side effects[21].

Mucuna pruriens is a famous Indian medicinal plant, tropical twinning herb, commonly known as velvet bean or cow-age or cowitch or alkushi[22]. L-dopa is a major constituent present in whole herb[23]. Different parts of the herb have been used in Ayurvedic research since ancient times owing to their outstanding therapeutic values and heal many diseases such as bone fractures, cough, dog-bite, madness, pain, pleuritis, ringworm, scorpion sting, snake-bite, sores and syphilis, as well as being anticholesterolemic, antiparkinson, antidiabetic, aphrodisiac, antiinflammatory and antimicrobial, it is also used for the treatment of menstruation disorders. constipation. edema, fever. tuberculosis. etc.[24][25]. The constituents bufotenin, choline beta-carboline were responsible and for antiepileptic and anti neoplastic activity. Non nutritive compounds that contribute to flavour colour[22,26]. Antibacterial activity of Mucuna pruriens methanolic extract was evaluated and well known wide spectrum activity against Gram positive Bacillus cereus, Staphylococcus and Gram negative Proteus vulgaris[27][28]. Mucuna cotyledon powder's pruriens antiparkinson activity resulted in a substantial improvement in brain mitochondrial complex-I activity but had no effect on overall monoamine oxidase activity (in vitro) despite the presence of NADH and coenzyme Q-10 in the cotyledon powder[29]. In vitro tests revealed that an ethyl acetate whole plant extract and a methanolic extract of Mucuna pruriens, all of which contain significant quantities of phenolic compounds, had high antioxidant and free radical scavenging activities. These plant extracts served as a significant source of natural antioxidants, which might be helpful in preventing the progress of various oxidative stresses (Jimoh et al. 2020)[30]. Soaking, frying, dehulling, drying, and milling into flours are some of the physical and biochemical processes used to process M. pruriens beans[31]. The aqueous extract of M. pruriens seeds (100 and 200 mg/kg body weight) substantially decreased blood glucose levels 2 hours after oral administration in normal and Streptozotocin diabetic rats. They explained that this hypocholestric activity is due to the presence

of squalene content[32]. Our team has extensive knowledge and research experience that has translated into high quality publications[33–44]. [45–49].

The current study evaluates the antiinflammatory properties of *Mucuna pruriens*, the green synthesis, characterization of CuNPs, and screening of their cytotoxic activity.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

Mucuna pruriens extract is purchased commercially. The extract is diluted with 100 ml of distilled water and boiled for 10-15min at 70 degree Celsius. The extract is then filtered using Whatman filter paper and allowed to stand for 40 min undisturbed. A 60 ml of filtered extract is obtained and used for green synthesis.

2.2 Synthesis of CuNPs

A 30 milli molar of copper (II) sulfate is weighed and mixed with distilled water of 100 ml and mixed with the 40 ml of filtered extract. The extract is permitted to stand in the stirrer for a duration of 1 h and kept in the shaker for intermixing of the particles to obtain green synthesis. The reduction of copper (II) sulfate to CuNPs was periodically monitored by ultravioletvisible (UV) spectrometers. UV-visible spectral analysis was done for an interval of every 2 h. After 3 days of synthesis, the extract is collected and centrifuged for 10 min. Nanoparticles are found to be settled down in the centrifuge tube. Filler and scrapers are used to remove the nanoparticles from the tube and stored at optimum temperature.

2.3 Anti-inflammatory Activity (Albumin denaturation Assay)

A 2 ml of 1% bovine albumin fraction was mixed with 400 μ l of *Mucuna pruriens* mediated copper nanoparticles in different concentrations (10-50 μ g/mL) and the pH of 6.8 is adjusted using 1 N HCl. Incubation at room temperature is done for 20 min and then the mixture is heated at 55°C for 20 min in a water bath. The absorbance value was recorded at 660 nm after the mixture is cooled at room temperature. The standard that is used for the activity is diclofenac sodium in different concentrations. The experiment is performed in triplicate. Percentage of protein denaturation was determined utilizing following equation,

% Inhibition= Control O.D - sample O.D * 100/ control O.D

2.3 Cytotoxicity Assay (Brine Shrimp Lethality Assay)

Brine shrimp eggs are purchased. Artificial sea water is prepared in a bottle by dissolving 35 g of sodium chloride in 1 L of distilled water and the dried cysts are placed in them. Incubation is made at 37°C for 48 h under strong aeration and illuminations and the nauplii are hatched after the incubation period. The cytotoxicity activity of CuNPs in brine shrimp is evaluated. The experiment is performed in a 6-well plate containing artificial sea water and 10 nauplii. well Each incubated with different is concentrations of Mucuna pruriens mediated copper nanoparticles ranging from 5 µl, 10 µl, 20µl, 40µl, and 80 µl, respectively. The number of surviving shrimps is counted and taken into account after 24 h. The lethality concentration (LC50) of <100 ppm is considered as potent (active).

Percentage of Lethality = number of dead nauplii/ number of dead nauplii + number of live nauplii * 100.

2.4 Statistical Analysis

The results were statistically analysed by Chisquare test using SPSS software version 22. P value less than 0.05 is taken significant.

3. RESULTS

The results obtained are recorded and the percentage of inhibition is calculated for antiinflammatory activity and shown in table 1. It was found that values for Anti inflammatory properties of Cu Nanoparticles was lesser than the standard values at low concentrations. Percentage of inhibition was 17% at 10 µL concentration, 24% at 20 µL, 43% at 30 µL and 54% at 40 µL and highest at 50 µL (71%). The results of cytotoxic activity is shown in table 2. At 5 µL concentration there was a death of 10% of nauplii, at 10 µL there was a death of 20% of nauplii, at 20 µL and 40 µL there was a death of 30% of nauplii and at 80 µL there was a death of 40% of nauplii. It was seen that as the concentration increased, the percentage of lethality also increased.

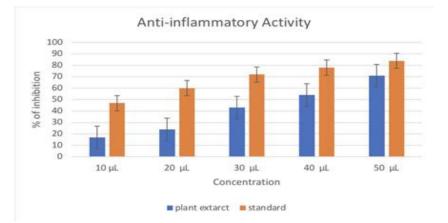
The bar graph 1 represents the comparison between the Mucuna pruriens (Cu Nps) and the standard diclofenac sodium. (Chi square test was analysed and p value was 0.220, and it was found to be statistically insignificant). The maximum percentage of inhibition is found to be 71% at 50 µl which is close to that of the standard drug Diclofenac sodium. The bar graph 2 represents the comparison between the percentage of lethality in Day-1 and Day-2. (Chi square test was analysed and p value was 0.242, was found to be and it statistically insignificant). The maximum percentage of lethality is found to be 40% at 80 µl than the control group.

Concentration (µI)	% of inhibition	Standard drug
10	17	47
20	24	60
30	43	72
40	54	78
50	71	84

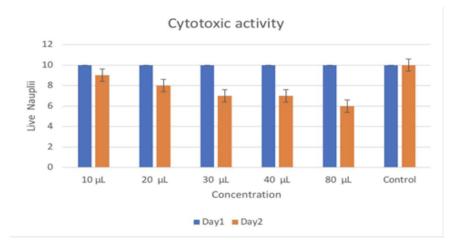
Table 1. depicts the anti-inflammatory property of Copper nanoparticles with Mucuna pruriens at various concentrations compared with the standard drug

Table 2. depicts the cytotoxicity of Copper Nanoparticles with Mucuna pruriens

Concentration (µI)	Viable Nauplii	% Death	
5	9	10	
10	8	20	
20	7	30	
40	7	30	
80	6	40	
Control group	10	0	



Graph 1. The bar graph represents the comparison between the *Mucuna pruriens* (Cu Nps) and the standard diclofenac sodium. X- axis represents the percentage of inhibition and Y- axis represents the concentration of the *Mucuna pruriens* (Cu Nps) and the standard diclofenac sodium. (*Chi square test was analysed and p value was 0.220, and it was found to be statistically insignificant*).The maximum percentage of inhibition is found to be 71% at 50 µl which is close to that of the standard drug Diclofenac sodium



Graph 2. The bar graph represents the comparison between the percentage of lethality in Day-1 and Day-2. The X- axis represents the Live nauplii and the Y- axis represents the concentration of the Mucuna pruriens (Cu Nps) and the control group. (*Chi square test was analysed and p value was 0.242, and it was found to be statistically insignificant*). The maximum percentage of lethality is found to be 40% at 80 µl than the control group

3. DISCUSSION

The anti-inflammatory activity of *Mucuna pruriens* mediated CuNPs is depicted in Graph-1. The percentage of inhibition of protein denaturation in bovine serum albumin increases simultaneously along with the increase in the concentration of CuNPs. The maximum percentage of inhibition is found to be 71% at 50 μ l which is close to that of the standard drug Diclofenac sodium. In previous studies, it was reported that Ethanolic extract of *M. pruriens* had been shown to significantly (P < 0.001) reduce carrageenan-induced paw edema

in rats at the early stage of inflammation resulting in suppression of histamines and serotonins[50]. Another studies, reported a high percentage reduction in edema size of between 45% and 50% when treated with seed powder of M. pruriens from Nigeria[51]. The present study shows that production of *M. pruriens* mediated Cu Nps at 660 nm was found to be significant anti inflammatory activity and can be used as a drug to modulate various inflammatory mediators such as cytokines, prostaglandins, NO, histamine, and serotonin.

Similarly, the cytotoxic activity of Mucuna pruriens mediated CuNPs is depicted in the graph represents. As the graph-2. the percentage of lethality increases as there is an increase in the concentration. The maximum percentage of lethality is found to be 40% at 80 µl. In previous studies, investigated the cytotoxic treatment of human cervix adenocarcinoma (HeLa) cells with crude extracts of M. pruriens seeds, however, showed that the seeds were not toxic at 50 μ g/mL, therefore suggesting that M. pruriens seeds are safe for human consumption[52]. Another study investigated the cytotoxicity of aqueous Mucuna pruriens L. leaf extract by doxorubicin on different human cancer cell lines. The highest cytotoxic activity of the test extract was observed in HeLa cells at halfmaximal inhibitory concentration (IC50) = 92.8 µg/ml[52,53]. From this present study, cytotoxic activity test of Mucuna pruriens mediated Copper Nanoparticles indicates the decreased cytotoxic activity exhibited by the Mucuna pruriens Copper Nanoparticles in lesser mediated concentrations. Hence, this shows that dose dependent formulations of Mucuna pruriens mediated Copper Nanoparticles which prove to be less toxic and safe for therapeutic treatments.

Based on the findings of the present investigation we can say that reinforcing copper nanoparticles with Mucuna pruriens has a synergistic effect and can be used as an alternative to commercially available anti-inflammatory and chemotherapeutic agents. Limitations of this research include the fact that it was conducted in vitro and we didn't evaluate it by other standard tests like membrane stabilization and anti lipoxygenase activity for anti inflammatory activity as well as MTT assay for cytotoxic activity. These nanoparticles may be used in the future to design and target novel medications, as well as provide care for a variety of acute and chronic diseases with reduced side effects. As well as, the confirmed lowered cytotoxic effect of Mucuna pruriens mediated copper nanoparticles provides a potential application of these in later. Hence, these Mucuna pruriens (Cu Nps) formulations were seen to have biocompatibility, as well as high potential for application in the fields of medicine and food.

4. CONCLUSION

The use of herbal medicine is growing in order to be compatible with the promise and capabilities of curing diseases. Herbal drug mediated nanoparticles have a major activity that has no side effects and is safe to consume. Based on the results of the current study, it is concluded that *Mucuna pruriens* mediated Cu Nps can be used as a potential source of anti inflammatory agent due to inhibition of AA metabolism, COX, LOX, cytokines and NF-kB and also as an anti cancer drug for the treatment of tumours and cancers.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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