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Nanotechnology Improves the Activity of Herbal Medicines: Green Synthesis of Silver Nanoparticles Using *Costus Afer* Leaf Extract and its Effect on Hematological Indices in Adult Male Wistar Rats.

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ABSTRACT

Nanotechnology is a rapidly growing area of scientific interest due to its wide applications in catalysis, solar energy, waste management, and sensing technology. Nanomaterials are efficiently used in the field of medicine for the purpose of drug delivery, diagnosis, treatment of cardiovascular diseases, wound healing, and development of antimicrobial agents. The present study aimed to evaluate the effect of *Costus afer*-AgNPs extract on the PCV level of male rats. In this experimental study, 18 adult male rats were used. The rats were randomly divided into six groups. The first group (control) adequately consumed compressed food and water without any restrictions during the experiment. The second, third, fourth, fifth, and sixth experimental groups respectively consumed 200mg, 400mg, 600mg, 800mg, and 1000mg *Costus afer*-AgNPs extract per kilogram body weight in a daily manner. Blood samples were taken from all groups after four weeks through anesthesia. PCV and Hb test was carried out using the blood serum. The collected data were analyzed using SPSS, ANOVA, and LSD tests. PCV levels significantly increased in the experimental groups receiving 600mg, 800mg, and 1000mg *Costus afer*-AgNPs extract per kilogram body weight compared to the control group ($P < 0.05$). *Costus afer*-AgNPs maintained PCV levels. Hb concentration significantly increased in the experimental groups receiving 600mg, 800mg, and 1000mg *Costus afer*-AgNPs extract per kilogram body weight compared to the control group ($P < 0.05$). *Costus afer*-AgNPs increased Hb concentration. This study's findings suggest that *Costus afer*-AgNPs extract could prevent anaemia by possessing a blood tonic effect.

KEYWORDS: Nanoparticle, Silver, Medicinal plant, *Costus afer*, Haematology

INTRODUCTION

Due to the unique properties of nanotechnology, its application in medicine has made a great revolution in the fields of health for its ability to improve some medical diagnoses as well as treat and prevent diseases (Arshad *et al.*, 2021; Li *et al.*, 2021; Sahu *et al.*, 2021; Zhang *et al.*, 2021). Among the metallic nanoparticles are silver nanoparticles (AgNPs) that have antimicrobial, catalytic, and other properties, which are firmly involved in medicine and pharmaceutical applications (Ozdan, 2021). On the other hand, there are growing concerns about the toxicity of nanoparticles to human health (Ozdan, 2021). Nanotoxicology is a modern scientific branch that aims to clarify the potential negative effects of nanoparticles and related parameters affecting the cytotoxicity of nanomaterials (Ozdan, 2021).

The use of plants in medicine is not limited or restricted to any region of the world. It is an age-long practice in various parts of the globe for both preventive and curative purposes. Dependence on herbs as medicine in the treatment of diseases is still much practiced by a large proportion of the rural populace because of its ready availability and affordability (Sani *et al*., 2009). The long history of clinical application and natural origin guarantee that herbal products are effective and non-toxic (Shin *et al*., 2009). Recently, concerns have been raised over the lack of quality control and scientific evidence for the efficacy and safety of medicinal plants (Firenzuoli and Gori, 2007; Rousseaux and Schachter, 2003). Several warnings have been issued regarding the potential adverse effects of herbal remedies including hepatotoxicity and nephrotoxicity (Wojcikowski *et al*., 2004; Stickel *et al*., 2005; Seeff, 2007; Tang *et al*., 2008). Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively, or in synergy to improve health (Azaizeh *et al*., 2003; Gurib-Fakim, 2006). Bitters, for example, are known to stimulate digestion while phenolic compounds could be responsible for the anti-inflammatory and anti-oxidative activity of plant extracts. There is continuing interest in the evaluation of natural products as potential chemotherapeutic agents. This is encouraged by the isolation of phytochemicals in plants which could become important drugs in modern medicine (Wintola *et al*., 2010). Plants produce bioactive compounds which act as defense mechanisms against predators and at the same time, may be toxic in nature (Da Roch *et al*., 2001; Bent and Ko, 2004). With the upsurge of interest in medicinal plants, there is need for thorough scientific investigations of these plants for both efficacy and potential toxicity (Ashafa *et al*., 2010)

Costus afer (*C. afer*) is a plant commonly known as the ginger lily, spiral ginger, or bush cane. It is reportedly used in traditional medicine practice (TMP) to treat and manage many ailments including diabetes mellitus, stomach ache, arthritis, inflammation, and gout (Boison *et* al., 2019). These purported ethnomedicinal uses have triggered many research studies on the plant to amass scientific evidence. The search report of Boison et al. (2019), revealed that the stem and leaves of the plant contain substantial amounts of micronutrients and macronutrients. The leaves, stem, rhizomes, and roots of *C. afer* contain several steroidal sapogenins, aferosides, dioscin, and paryphyllin C and flavonoid glycoside kaempferol-3-O-α-L-rhamnopyranose. Experimental studies on various parts of the plant showed bioactivities such as antihyperglycemic, hepatocellular protection, cardioprotection, nephroprotection, testicular protection, CNS depressant, analgesic, antiarthritis, antibacterial, and antioxidant (Boison *et* al., 2019).

Many studies reported that oral ingestion of medicinal drugs can alter the hematological parameters ranges to either positive or negative. However, many of these therapeutic effects have been confirmed by contemporary scientific research and their antistress effects have not been well-researched (Ramadan *et al* ., 2015). It is widely known that Silver nanoparticles (Ag-NP) are used as antimicrobial substances. The use of Ag+ ions causes damage to many microorganisms. Different studies have shown that nanostructures especially nanoparticles, nanorods, and nanotubes, cause hemolysis and blood clotting (Al-Baker *et* al., 2020). Despite the extensive use of the plant, much work has not been done to study some of the toxicological implications on other related systems. Anyhow, it is reported that, due to the large surface area, a significant increase in vitro hemolysis was observed with AgNPs compared with micron-sized particles (Al-Baker *et* al., 2020). Hence this problem has led to the present study. Almost every part of this *Costus afer* is endowed with medicinal potential in diseases such as malaria, measles, diabetes mellitus, arthritis, and stomach disorders. In West Africa for instance, the succulent stem is chewed to quench thirst and also to treat cough and its accompanying sore throat. Various solvent extracts of the plant leaves, stems, rhizomes, and roots have been studied and reported to contain chemical compounds that could be useful in the alleviation of oxidative stress-related conditions. However, leads from traditional nutritional and medicinal practices have proven that some medicinal plants and edible vegetables may be effective in controlling and treating ailments with minimal side effects, as an alternative therapy, especially in developing countries. Although these plants have been used for the traditional management of various ailments, not many such medicinal plants like *Costus afer* have been scientifically validated. Therefore, there is a need to evaluate the effect of its synthesized leaf extract on the hematology of albino rats and equally to characterize the synthesized nanoparticles.

MATERIALS AND METHODS

Plant Materials

The leaves of *Costus afer* was collected from a farm in Umuaduru in Osisioma L.G.A of Abia State Nigeria. The plant sample was identified by a Botanist, Dr. Duru, C.N. of Environmental Biology Federal Polytechnic Nekede.

Animals

Adult male rats were used for this study. These animals were purchased from a local breeder in Ihiagwa Owerri-West L.G.A of Imo State. The animals were kept in well aerated stainless steel wire cages in the animal house of the Department of Biochemistry. The rats were given standard feed for at least two week after purchase to acclimatize them to laboratory environment before use.

Chemicals and Reagents

Chemicals

All chemicals and reagents used in this study was of good and analytical grade.

Methods

Preparation of plant material and Extraction

Fresh and healthy leaves were collected locally and rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature. About 10 g of these finely incised leaves of each plant type were weighed separately and transferred into 250 mL beakers containing 100 mL distilled water and boiled for about 20 min. The extracts were then filtered thrice through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) in 250 mL Erlenmeyer flasks for further experiments. In each and every steps of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

Synthesis of Silver Nanoparticles (Ag-np)

The green synthesis of Ag-np was prepared following the method reported in the literature (Khan *et al*., 2018). Preparation was done by reacting 10 mL of the *Cucumis sativus* fruit extract with 90 mL AgNO3 solution (1 mM) and was agitated on the air bath magnetic stirrer for 15 minutes at room temperature. A colour change was observed from colourless to pink. The mixture was centrifuged and dried in the oven at temperature between 50°C – 60°C overnight

Experimental design

The experimental animals were randomized into 3 groups of 5 rats each and treated as follows;

Group 1: The rats in this group served as control and were given normal diet and distilled water.

Group 2: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 200 mg/kg (Low Dose) to the rats in this group.

Group 3: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 400 mg/kg (low Dose) to the rats in this group respectively via oral gavage daily for 28 days.

Group 4: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 600 mg/kg (High Dose) to the rats in this group respectively via oral gavage daily for 28 days.

Group 5: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 800 mg/kg (High Dose) to the rats in this group respectively

Groups	No of Rats	PCV (%)
Normal Control	3	44.53 ± 0.42^{c}
200mg/kg bwt	3	40.25 ± 0.35^{a}
400mg/kg bwt	3	42.05 ± 0.07^{b}
600mg/kg bwt	3	41.76 ± 0.07^{b}
800mg/kg bwt	3	45.01 ± 0.01^{d}
1000mg/kg bwt	3	46.76 ± 0.01^{e}

via oral gavage daily for 28 days.

Group 6: Synthesized nanoparticle of *Costus afer* mediated AgNps was administered orally at 1000 mg/kg (High Dose) to the rats in this group respectively via oral gavage daily for 28 days.

Body weight and organ weight measurements

The body weights of the rats were taken weekly while their organ weights were taken at the end of the experiment (after sacrifice) using a Top loader weighing balance.

Sacrifice of animals

At the end of 28 days, a transverse incision was made through the ventral wall of the abdomen of each rat under slight chloroform anaesthesia. Blood samples was also obtained from the descending abdominal aorta and homogenized in a plain bottle for hormonal assay estimation.

Hematological estimations

The hematological indices (PCV, and Hb) will be assayed by the method outlined by Dacie and Lewis (2001).

Determination of Packed Cell Volume

Principle: When whole blood sample is subjected to a centrifugal force for maximum RBC packing, the space occupied by the RBCs is measured and expressed as percentage of the whole blood volume.

Method: Using microhaematocrit method, a well-mixed anticoagulated whole blood was allowed to enter capillary haematocrit tubes until they were approximately 2/3 filled with blood. Blood filling was done for each tube. One end of each tube was sealed with plastacine and placed in the medial grooves of the haematocrit centrifuge head exactly opposite each other, with the sealed end away from the centre of the centrifuge. All tubes were spun for five minutes at 1000rpm. The tubes were removed as soon as the centrifuge had stopped spinning.

Calculation: PCV was obtained for each tube using microhaematocrit-reader by measuring the height of the RBC column and expressing this as a ratio of the height of the total blood column.

$$\text{PCV (\%)} = \frac{\text{Height of cell column}}{\text{Height of total blood column}} \times 100$$

Determination of Haemoglobin (Hb) Concentration

Principle: When whole blood is added to Drabkin's reagent: a solution containing KCN and $K_3Fe(CN)_6$, KCN converts Hb-Fe^{2+} (ferrous) to Hb-fe^{3+} (ferric) state to form methaemoglobin which then reacts with KCN to form a stable pigment, cyanmethaemoglobin complex. The colour intensity of this mixture is measured in a spectrophotometer at a wavelength of 540nm (or using a yellow-green filter). The optical density (OD) of the solution is proportional to the haemoglobin concentration. All forms of Hb (Hb-C, Hb-O, etc) except Hb-S are measured with this cyanmet-method.

Method: Exactly 5.0ml of Drabkin's reagent was pipetted into two test tubes 1 and 2 and a well-mixed sample of EDTA blood (0.02ml) was pipetted into the tubes, rinsing the pipette five times with the reagent, until all the blood was removed from the pipette. The solutions were well mixed and allowed to stand at 250°C for 10 mins in order to allow the formation of Cyan-met-haemoglobin. The mixtures were transferred into cuvettes and read in a

spectrophotometer at a wavelength of 540nm. The Drabkin's reagent in tube 1 was used as the blank (setting the percentage transmittance at 100 %). The readings from each tube was recorded and the actual Hb values in g/dl were determined from a pre-calibrated chart.

Calculation:

$$\text{Hb in g/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. of standard (in mg/dl)}$$

Statistical Analysis

Values will be represented as Mean ± SD. Data obtained will be subjected to one way Analysis of Variance (ANOVA) and group means were compared using Duncan's new multiple range tests. Differences were considered to be significant at ($p \leq 0.05$).

RESULTS AND DISCUSSION

Effect of *Costus afer*-AgNps on PCV level of experimental rats.

Table 1.: Result showing the *Costus afer*-AgNps on PCV level of experimental rats

n = 3. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across columns are considered non-significant ($p > 0.05$)

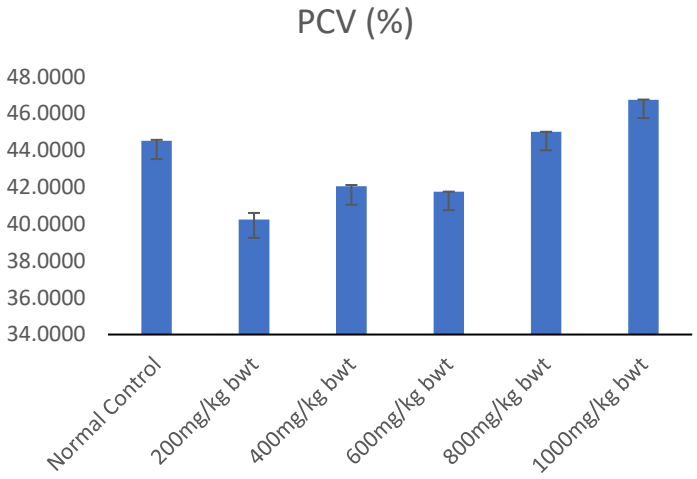

Figure 1: Graph showing the effect of *Costus afers*-AgNps on the PCV level of experimental rats.

Effect of *Costus afer*-AgNps on Haemoglobin concnetration of experimental rats.

Table 2: Result showing the *Costus afer*-AgNps on Hb concentration of experimental rats

Groups	No of Rats	Hb
Normal Control	3	15.03 ± 0.04^{b}
200mg/kg bwt	3	13.84 ± 0.05^{a}
400mg/kg bwt	3	15.65 ± 0.07^{d}
600mg/kg bwt	3	16.29 ± 0.14^{f}
800mg/kg bwt	3	15.31 ± 0.07^{c}
1000mg/kg bwt	3	16.16 ± 0.01^{e}

n = 3. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across columns are considered non-significant ($p > 0.05$)

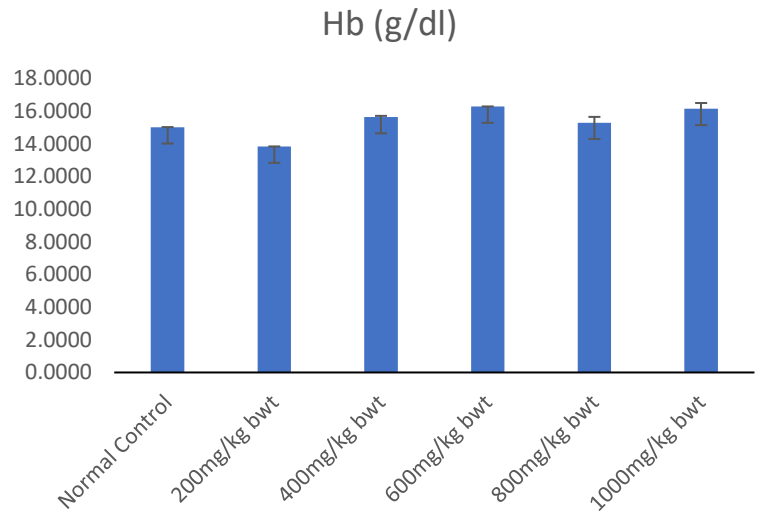

Figure 2: Graph showing the effect of *Costus afers*-AgNps on the Hb concentration of experimental rats.

Medicinal plants are of great importance to the health of individuals and communities and their medicinal values lie in some chemical substances that produce definite physiological actions on the human body. Blood parameters are good indicators of physiological and nutritional status of animals. Changes in haematological parameters have been used to elucidate the impact of nutritional factors and or additives supplied in diets of living organisms (Majid *et al*., 2010). They can also be used to explain blood relating functions of chemical compounds including plant extracts (Yakubu *et al*., 2007).

The extract showed a significant effect on the packed cell volume of the rats in the experimental groups when compared with the control during the initial 28 days of treatment. Packed cell volume (PCV) is a measure of the portion of the blood volume that is made up by red blood cells. The significant effect of the extract of *Costus afers*-AgNps at 200, 400, 600, 800 and 1000mg/kg body weight on the PCV throughout the experimental period is an indication that there was no destruction of matured cells and there was a change in the rate of blood cells production (haematopoiesis). Since low dose and high dose of the extract significantly increased the packed cell volume (PCV) of the control, the extract could be attributed to be non-toxic at the doses investigated and could have a protective effect against anemia.

It was observed that there was significant increase ($P < 0.05$) in the haemoglobin concentration of rats in the experimental groups when compared with the control after the initial twenty-eight (28) days of treatment. *Costus afers*-AgNps has shown to contain substantial amounts of essential amino acids and iron. The increase in the haemoglobin concentration could be attributed to the presence of these amino acids and high iron content. Iron is an important component of haemoglobin and functions in the transport of oxygen to cells and tissues. Haemoglobin concentration of rats in groups II treated with 200mg dose of the extract were significantly lower ($P < 0.05$) compared to the control group, but group V treated with a higher dose of the extract at 800mg was significantly lower ($P < 0.05$) on comparison with the other test groups. This is an indication that the *Costus afers*-AgNps could be a better haematinic agent and possess a blood tonic effect. Nevertheless, findings confirms that plants have medicinal properties which make them of this shrub in maintaining healthy blood glucose levels useful for the treatment of some diseases (Ali *et al*., 2016). Scientific studies have established traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly increasing in industrialized countries.

Conclusion

The compounds contained in extracts of *Costus afer* leaf mediated with synthesized silver particles effectively increased the PCV level of experimental rats and also effectively increased the Hb of experimental rats. Conclusively, the AgNps mediated with *Costus afer* could be used to manage anemic-related conditions since it could cause a significant increase in the Hb and PCV levels. Further investigations should be carried out to isolate active molecules and validate the therapeutic potential of the plant in the management of chronic diseases like diabetes, hypertension and cancer. Since a study revealed that the in-take of the extract could lead into excessive regeneration of cells i.e hyperplasia, further research should be carried out to know if these cells are cancerous.

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