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ASSESSMENT OF ANTI-INFLAMMATORY ACTIVITY OF COPPER OXIDE NANOPARTICLES FROM *Sarcostemma acidum* STEM EXTRACT

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ABSTRACT

To meet the increasing demands for commercial nanoparticles new eco-friendly "green" methods of synthesis are being discovered. Plant mediated synthesis of nanoparticles offers single step, easy extracellular synthesis of nanoparticles. Nanotechnology refers broadly to a field of applied science and technology whose unifying theme is the control of matter on the atomic and molecular scale. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The silver nanoparticles have various and important applications. In this work, we describe a cost effective and environment friendly technique for green synthesis of copper oxide nanoparticles through the extract of *Sarcostemma acidum* stem as it is acts as a reducing as well as capping agent. The synthesized copper oxide nanoparticles exhibited potential anti-inflammatory activity.

Keywords: Nanochemistry, *Sarcostemma acidum*, Copper oxide nanoparticles, Anti-inflammatory activity

INTRODUCTION

Nanotechnology is becoming an innovative area of increasing research and industrial interest since the1980's. Nanotechnology can be defined as the manipulation of atom by atom from the material world by the combination of engineering, chemical and biological approaches. In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. Most of the current strategies are usually working by the use physical or chemical principles to develop a myriad of nano-objects with multiple applications. Main fields of nanotechnology applications range from catalysis, micro- and nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis and others **[1]**. Nanochemistry is relatively new areas of science arisen in last decade of past century after discovery of fullerenes and nanotubes. It is introduced into more extent interdisciplinary integrated modern science known now as rapidly developing nanotechnology **[2]**.

Metal nanoparticles can be prepared by physical, chemical and biological routes; the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach in which the metal ions in solution is reduced in conditions favoring the subsequent formation of small metal clusters or aggregates **[3]**. Various metals like copper, titanium, gold, silver and iron were used for the synthesis of nanoparticle. Among the noble metals, copper nanoparticles have become the focus of intensive research due to its wide ranges of application for many sectors of life and industry **[4]**. Recently, biosynthetic methods employing naturally occurring reducing agents such as polysaccharides, biological microorganism such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex physical and chemical synthetic procedures to obtain CuONPs **[5]**.

In the recent decades, increased development of green synthesis of nanoparticles is inevitable because of its incredible applications in all fields of science. There were numerous work have been produced based on the plant and its extract mediated synthesis of nanoparticles. CuONPs has been synthesized by using the plant broth from a wide variety of plants such as *Zingiber officinale* **[6]** and *Adathoda vasica* **[7]**. Keeping in view, in the present study to evaluate the anti-inflammatory activity of copper oxide nanoparticles using *Sarcostemma acidum* stem.

MATERIALS AND METHODS

Preparation of extract

Sarcostemma acidum aqueous extract was prepared by placing 10 g of dried fine powder in 500 ml glass beaker along with 400 ml of sterile distilled water. The mixture was boiled for 10 min until the colour of solution changed from watery to brown-yellow. Then the mixture was cooled to room temperature and filtered with Whatman No. 1 paper, before centrifuging at 1200 rpm for five minutes to get rid of biomaterials. The extract was stored at room temperature in order to be used for further experiments.

Copper oxide nanoparticle (CuONPs) synthesis

In the synthesis of copper oxide nanoparticles, 2.8g of copper acetate monohydrate was dissolved in 500 ml of the deionized water and stirred magnetically at room temperature for 5 minutes. *Sarcostemma acidum* stem aqueous extract were added dropwise under magnetic stirring and the blue color of copper ions change to green color, when the extract comes in contact copper ions. The acquired green mixture was kept at room temperature. After 10 minutes, the green mixture started altering to a brown suspended mixture, representing the development of water soluble monodispersed copper oxide nanoparticles **[8]**.

In vitro anti-inflammatory activity

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra *et al*. **[9]**.

RESULTS AND DISCUSSION

Synthesis of copper oxide nanoparticles

Our earlier report showed that Copper oxide nanoparticle synthesized from copper acetate monohydrate using aqueous extract of *Sarcostemma acidum* stem. Spherical and polydispersity of Copper oxide nanoparticle of particle sizes ranging from 32 – 82 nm with an average size of 56.76 nm were obtained **[10]**.

Anti-inflammatory activity

The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair, which are aimed at host, defense and usually activated in most disease condition **[11]**. Chronic inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. At present, although synthetic drugs are dominating the market, element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration **[12]**. Currently much interest have been paid in the search of nanoparticle with anti-inflammatory activity which may lead to the discovery of new therapeutic agent that is not only used to suppress the inflammation but also used in diverse disease conditions where the inflammation response amplifies the disease process. There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of *Sarcostemma acidum* stem extract and CuONPs. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins (Egg albumin and Bovine serum albumin) *in vivo* **[13, 14]**. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein denaturation by *Sarcostemma acidum* stem extract, CuONPs and reference drug diclofenac sodium. The present findings exhibited a concentration dependent inhibition of protein denaturation by the *Sarcostemma acidum* stem extract and CuONPs **(Table 1 and Figure 1)**. The lowest activity of *Sarcostemma acidum* stem extract, CuONPs and Diclofenac sodium were 19.17, 21.91, and 23.28% in the concentration of 10µg/ml respectively while the highest activity of *Sarcostemma acidum* stem extract, CuONPs and Diclofenac sodium were 79.45, 86.30, and 93.15% in the concentration of 100µg/ml respectively. The greatest effect of CuONPs (100 µg/ml) was found to be near to standard diclofenac sodium. The half inhibition concentration (IC_{50}) of *Sarcostemma acidum* stem extract, CuONPs and diclofenac sodium were 59.47, 56.30, and 48.37$\mu g/ml^{-1}$ respectively. From the present study it can be concluded that CuONPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein **(Table 1 and Figure 1)**. Our result agrees with the earlier report **[6, 7]**.

The present findings exhibited a concentration dependent inhibition of protein

(Bovine serum albumin) denaturation by the *Sarcostemma acidum* stem extract and CuONPs **(Table 2 and Figure 2)**. The lowest activity of *Sarcostemma acidum* stem extract, CuONPs and Diclofenac sodium were 19.20, 20.46 and 22.24% in the concentration of 10µg/ml respectively while the highest activity of *Sarcostemma acidum* stem extract, CuONPs and Diclofenac sodium were 83.00%, 88.45% and 94.85% in the concentration of 100µg/ml respectively. The half inhibition concentration (IC_{50}) of *Sarcostemma acidum* stem extract, CuONPs and Diclofenac were 57.83, 53.27$\mu g/ml^{-1}$ and 47.37$\mu g/ml^{-1}$ respectively. The greatest effect of CuONPs (100 µg/ml) was found to be near to standard diclofenac sodium. From the present study it can be concluded that CuONPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein **(Table 2 and Figure 2)**. Our result agrees with the earlier report **[6, 7]**.

Table 1: Effect of *Sarcostemma acidum* stem extract, CuONPs and Diclofenac sodium on protein denaturation (Fresh egg albumin)

Concentrations (µg/ml)	% of inhibition		
	Sarcostemma acidum	**CuONPs**	**Diclofenac sodium (Standard)**
20	19.17±1.34	21.91±1.53	23.28±1.62
40	32.87±2.30	35.61±2.49	42.46±2.97
60	54.79±3.83	57.53±4.02	63.01±4.41
80	65.75±4.60	69.86±4.89	79.45±5.56
100	79.45±5.56	86.30±6.04	93.15±6.52
IC_{50} (µg/ml)	59.47	54.79	48.37

Values are expressed as Mean ± SD for triplicates

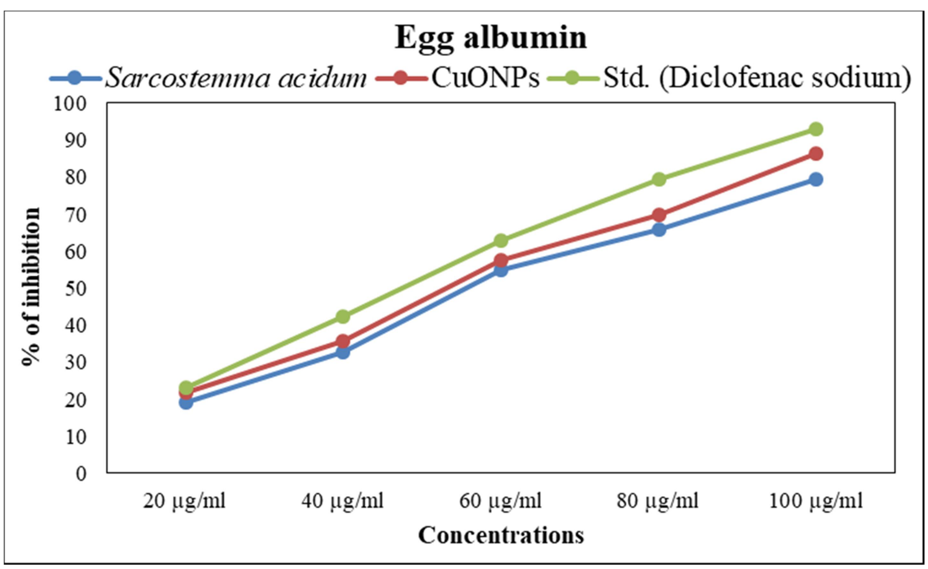

Figure 1: Effect of *Sarcostemma acidum* stem, CuONPs and Diclofenac sodium on protein denaturation (Fresh egg albumin)

Table 2: Effect of *Sarcostemma acidum* stem, CuONPs and Diclofenac sodium on protein denaturation (Bovine serum albumin)

Concentrations (µg/ml)	% of inhibition		
	Sarcostemma acidum	CuONPs	Diclofenac sodium (Standard)
20	19.20±1.34	20.46±1.43	22.24±1.55
40	33.05±2.31	38.92±2.72	46.06±3.22
60	54.56±3.81	59.60±4.11	64.42±4.50
80	69.04±4.83	72.50±5.07	78.48±5.49
100	83.00±5.81	88.45±6.19	94.85±6.63
IC_{50} Value (µg/ml)	57.83	53.27	47.37

Values are expressed as Mean ± SD for triplicates

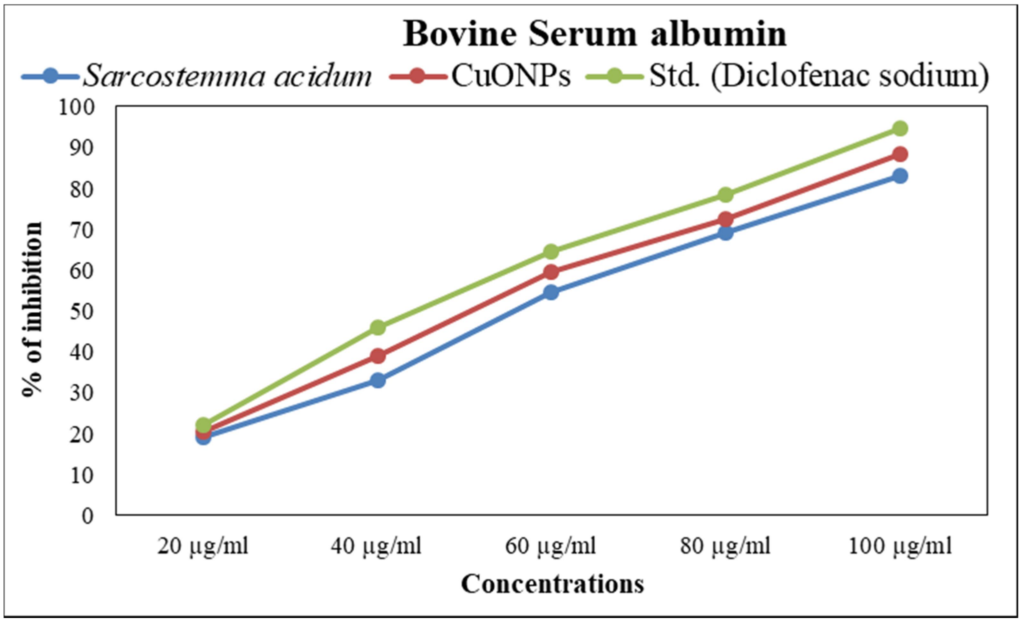

Figure 2: Effect of *Sarcostemma acidum* stem, CuONPs and Diclofenac sodium on protein denaturation (Bovine serum albumin)

CONCLUSION

Medicinal plants have medicinally important compounds in their different parts. The synthesis of nanoparticles using plants depends on the nature of plant such as its phytochemical content, special adaptation, and medicinal importance. In this study, we investigated eco-friendly and cost- effective green synthesis of silver nanoparticles using leaf extract of medicinal plant *Sarcostemma acidum* stem. The synthesized copper oxide nanoparticles exhibited anti-inflammatory activity. This finding suggests that the synthesis of CuONPs using *Sarcostemma acidum* stem extract could be a good source for developing green nano-medicine for the management of inflammation.

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