

Green Nanotech Enhancement in the Bio-Efficacy of Homeopathic Drug: Homeopathy Links with Modern Science

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ABSTRACT

Phyto-compounds facilitated bio-fabrication of nanoparticles has received an unprecedented significance for the enhancement of bio-efficacies of allopathic drugs. This enthusiasm has motivated us to investigate Green Nanotechnological enhancement in the bio-efficacy of homeopathic drugs using gold nanoparticles embedded with biomolecules present in homeopathic mother tincture. The present study warrants the synthesis of gold nanoparticles using *Carica papaya* homeopathic mother tincture in a rapid single green process. Gold nanoparticles embedded with *Carica papaya* homeopathic mother tincture (CpAuNps) have been thoroughly characterized for their optical property, surface morphology, crystalline lattice, particle size, and zeta potential using UV-Vis spectroscopy, FE-SEM, XRD and DLS and monitored for the enhancement in the platelets and Complete blood counts. Clinical trials (as per regulations laid down by Central Council of Homoeopathy, N. Delhi) have been performed on 30 patients (Age: 15-20 years) suffering from dengue fever, admitted in Faculty of Integrated Medicine Homoeopathic College & Hospital. Their blood samples have been collected at regular intervals under the supervision of the Medical Officer. Blood smears have been prepared at every alternate day to evaluate hematological parameters (platelet count) and CBC. The treatment of CpAuNps has depicted statistically significant enhancement ($p < 0.05$) in platelet counts and effective in maintaining CBC in normal range. The enhancement in the platelets and maintenance of CBC may be ascribed to the activation of the gene expression of ALOX12 (arachidonate 12-lipoxygenase or platelet-type lipoxygenase) and PTAFR (platelet-activating factor receptor gene) enhancing the number of megakaryocytes. Such metabolic changes are likely due to the result of induced green nano technological modifications involving high surface area to volume ratio, biocompatibility and astonishing optical properties related to surface plasmon resonance. Overall, phenomenon allows accumulation and penetration of nano CpAuNps into the living tissues comparatively deeper.

Keywords: Homoeopathic mother tincture, Green synthesis, CpAuNps, Enhancement in platelets and CBC

Abbreviations: CpAuNps: Gold Nanoparticles Embedded with *Carica papaya* Homeopathic Mother Tincture; CpMT: *Carica papaya* Homeopathic Mother Tincture; UV-Vis: Ultra Violet-Visible; FE-SEM: Field Emission Scanning Electron Microscope; XRD: X-Ray Diffraction; JCPDS: Joint Committee on Powder Diffraction Standards; DLS: Dynamic Light Scattering; CBC: Complete Blood Counts; ALOX12: Arachidonate 12-Lipoxygenase or Platelet-Type Lipoxygenase; PTAFR: Platelet-Activating Factor Receptor Gene

INTRODUCTION

Modern scientists do not have much faith in curative actions of homeopathic drugs and many of them consider it a placebo effect. Among various reasons, their action in extreme dilutions at which trace of physical matter present in homeopathic medicines can be measured, the biological mechanism of action, difficulty in conducting clinical trials and perhaps slow action. No doubt the biochemical pathways of homeopathic drugs are not very well understood as in allopathic medicines. However, the presence of any physical material in extreme homeopathic dilutions is

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increasingly understood based on the trace quantity of the starting material being retained in nanoforms.

In recent years, the bio-fabrication of noble metal nanoparticles has become an area of sustained research pertaining to the development of environmentally benign processes and products in biomedical research [1,2]. Nanoparticles are more bioavailable and biologically active forms of their source material [3,4]. Among the noble metals, gold and silver are attracting focus owing to their inherent medicinal properties [5-8]. Such metal nanoparticles may be synthesized by several physicochemical methods like spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis [9]. However, their biomedical applications are often restricted for the cost involved and associated toxicity issues. Various reports are available on the synthesis of noble metal nanoparticles using microbes and plant materials as nano factories [10-13]. The application of plant extracts for the synthesis of nanoparticles is increasingly appreciated because of their relatively less toxic and environment-friendly attitude [14,15]. The symbiosis between basic principles of green chemistry and nanotechnology has resulted in a new term Green Nanotechnology. Phyto-compounds facilitated synthesis of noble metal nanoparticles has received an unprecedented significance and widely applied for the enhancement in their bio-efficacies of allopathic nanodrugs [16]. Nanomedicine researchers have shown marked reductions of dose size and/or repetitions of nanoscale forms versus conventional bulk in the magnitude ranging from 10 to 1000 times lower [17,18]. Quantum dots and other very small nanomaterials can readily cross cell membranes. Their small size facilitates olfactory, oral, or dermal administration by allowing passive entry into cells and translocation via blood and lymph, including crossing the blood-brain barrier [19]. However, little efforts have been made towards the assessment of the capability of homeopathic mother tinctures of plant origin for the bio-fabrication of noble metal nanoparticles with enhanced medicinal activities [20]. In homeopathy, two categories of drugs are used, namely, mother tinctures and potentized forms. Mother tinctures are the dilute solutions of secondary metabolites derived from plants, animals or minerals using ethanol as extracting agent.

The present study warrants the synthesis of gold nanoparticles using *Carica papaya* a homeopathic mother tincture in a rapid single green process. Gold nanoparticles embedded with *Carica papaya* homeopathic mother tincture (*CpAuNps*) have been thoroughly characterized for their optical property, surface morphology, crystalline lattice, particle size, and zeta potential and monitored for the enhancement in the platelets and complete blood counts. The mother tincture of the homeopathic drug *Carica papaya* is the ethanolic extract of the fruits of the plant *Carica papaya* (papita) and is recommended as a

remedy for dengue fever. The enhancement in the platelets and CBC has been ascribed to the metabolic changes induced by green nanotechnological modifications involving high surface area to volume ratio, bio-compatibility, astonishing optical properties related to surface plasmon resonance and activation of the gene expression of *ALOX12* (arachidonate 12-lipoxygenase or platelet-type lipoxygenase) and *PTAFR* (platelet-activating factor receptor gene) enhancing the number of megakaryocytes. Such Overall, phenomenon allows accumulation and penetration of nano *CpAuNps* into the living tissues comparatively deeper.

MATERIALS AND METHODS

Carica papaya based homeopathic mother tincture (*CpMT*) was purchased from the homeopathic store of SBL, India. The synthesis of gold nanoparticles was carried out at a varied concentration of $\text{NaAuCl}_4 \cdot 2\text{H}_2\text{O}$ solution, keeping the concentration of *CpMT* constant as a function of pH.

Characterization of gold nanoparticle

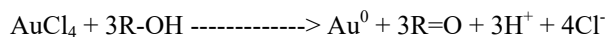
The gold nanoparticles were characterized for optical properties with the help of UV-Vis spectrophotometer (Lab India UV 3000+). The morphology of gold nanoparticles was studied using FE-SEM (Nova Nano FE-SEM 450, Netherland). The X-ray diffraction pattern of gold nanoparticles was recorded using XRD (Bruker AXS D8 Advance, Germany) over 30° - 80° with scan run 40/min, step size of 0.02° and Cu K α radiation of $\lambda=1.54 \text{ \AA}$. The hydrodynamic size distribution was analyzed using zeta sizer (Nano ZS90 model Malvern, Germany).

Estimation of platelets count and CBC

The present study was executed at DEI Faculty of Integrated Medicine, Homoeopathic Medical College, and hospital, Dayalbagh, Agra. The clinical trials (as per regulation laid down by Central Council of Homeopathy) were performed on 30 patients (Age: 15-20 years) suffering from dengue fever. Patients were divided into three groups (10 patients each) group. I was considered as a control group, group II was served *CpMT* and group III (*CpAuNps*: 1 ml - twice a day) for 7 days. Their blood samples were collected at regular intervals. Blood smears were prepared at every alternate day to evaluate hematological parameters (platelet count and complete blood count: *CBC*).

RESULTS AND DISCUSSION

Embedded gold nanoparticles (*CpAuNps*) were synthesized using the redox potential of secondary metabolites (glycosidic flavonoids) present in *CpMT*. The conversion of Au (III) to its elemental form (Au^0) was noticed by the visual change in color from pale yellow to ruby red.



Characterization of gold nanoparticle

Ultraviolet-visible spectrophotometer: The optimized experimental conditions of bio-fabrication of gold nanoparticles were as follows: *CpMT* (2 ml), sodium tetrachloroaurate dehydrate solution (3 ml; 1 mM) and

sonication (45 min; 50 KHz) at pH 6.8. It was found that at 2:3 ratio of *CpMT* and $\text{NaAuCl}_4 \cdot 2\text{H}_2\text{O}$ solution had a maximum absorption band at 543 nm (**Figure 1**). Any further dilution didn't show any marked increase in the absorbance, therefore, the ratio 2:3 was considered optimum.

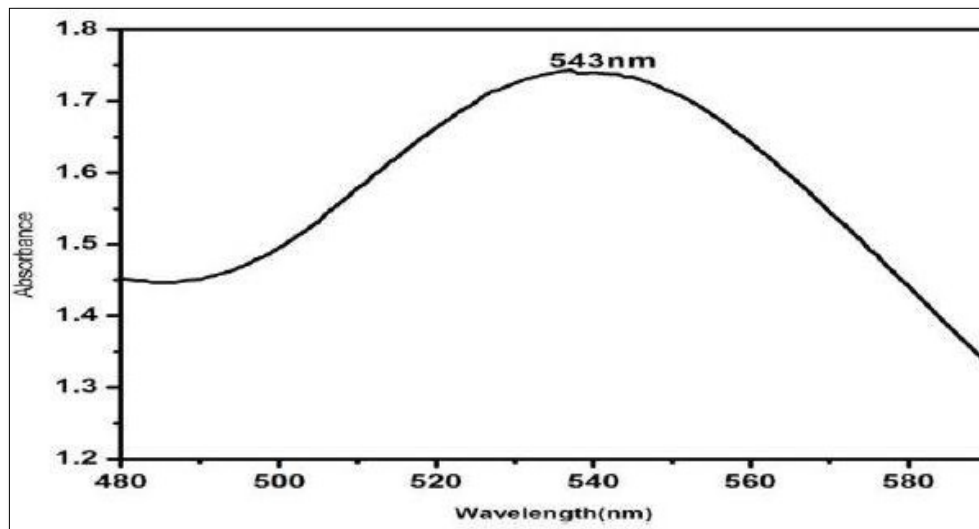


Figure 1. UV-Vis spectra of gold nanoparticles.

X-ray diffraction: Bragg diffraction peaks 2θ appeared at 38.18° , 44.3° and 64.9° in the embedded gold nanoparticles (**Figure 2**). It could be indexed to (111), (200) and (220) having lattice planes of face-center cubic compared with (JCPDS file 65-2870). The intensity of the diffraction peaks (200) and (220) were found lower than the corresponding crystallographic plane (111). The fact established that the

lattice plane (111) is the transcendent crystallographic plane and is more reactive because of its high atom density [21]. The observed noise and broadening of the peak in XRD record of Gold nanoparticle may be assigned to the existence of medicinally important secondary metabolites (flavonoids) loaded on the surface of bio-fabricated embedded gold nanoparticles.

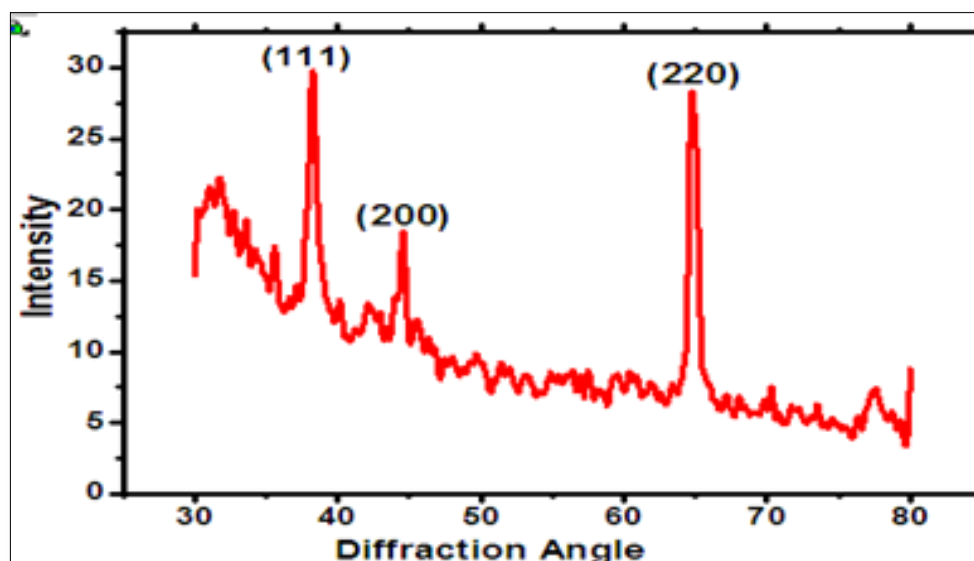


Figure 2. XRD graph of gold nanoparticles.

FE-SEM: FE-SEM images (**Figure 3**) acquired from the drop-coated film of nanoparticles indicated polydispersed

spherical shaped morphology of bio-fabricated gold nanoparticles.

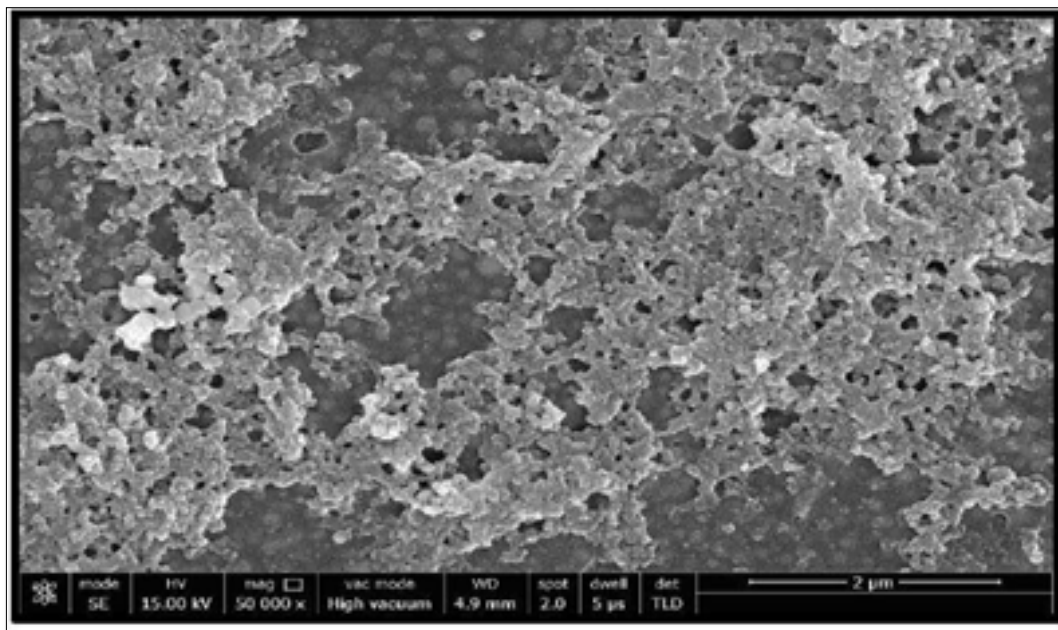


Figure 3. FE-SEM images of gold nanoparticles.

Dynamic light scattering: The dynamic light scattering (DLS) spectrum highlighted the asymmetric distribution of nanoparticles mainly in the range of 28 to 130 nm (**Figure 4a**). However, a little population was extended in the range of 1200 nm. The average hydrodynamic size (Z-Average) of embedded gold nanoparticles was found to be 62.8 nm.

Zeta potential of embedded gold nanoparticles determined in water medium as a dispersant was -10.9 mV (**Figure 4b**). The magnitude of observed high negative charge on the bio-fabricated nanoparticles might be acting as a repulsive barrier, avoiding aggregation of nanoparticles.

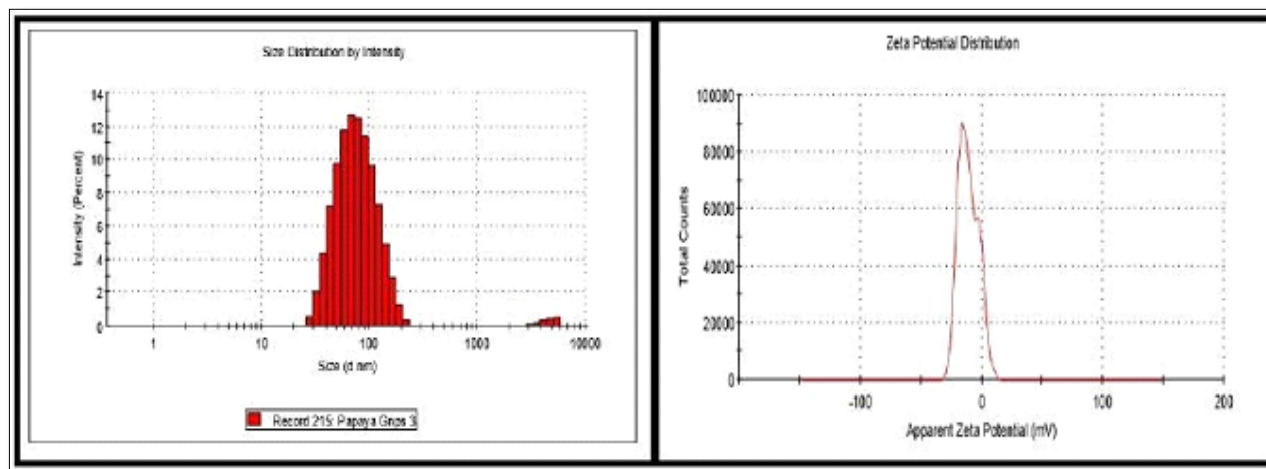


Figure 4. (a) Size distribution graph and (b) zeta potential of gold nanoparticles.

Estimation of platelets count and CBC

A perusal of **Table 1** depicts time-dependent (1st-7th day) increase in platelet count of normal control and *CpMT* and *CpAuNps* treated groups. However, in the normal control consisting of dengue patient group, there is an initial slight decrease in platelet count followed by a gradual increasing pattern but not to the level of platelet counts expected in a healthy person. Interestingly, about 50% enhancement in

the platelet counts has been observed in the case of *CpAuNps* compared to *CpMT* treated groups. An increasing trend (1st-7th day) has been observed in the case of *CBC* in normal control reaching to the value not expected for a normal health (**Table 2**). The treatment of *CpMT* and more effectively of *CpAuNps* has been found quite effective for maintaining *CBC* to a level of normal range (6,000-10,000 cells/ μ L).

Table 1. Platelet count of normal control, *CpMT* and *CpAuNps* treated groups.

Parameters	Groups	Day 1	Day 3	Day 5	Day 7
Platelet count (cells/ μ L)	Normal Control	47,261 \pm 0.15	42,850 \pm 0.13	46,357 \pm 1.17	53,245 \pm 1.07
	<i>CpMT</i>	46,380 \pm 0.16	50,900 \pm 0.16	1,23,850 \pm 0.81	1,92,624 \pm 0.98
	<i>CpAuNps</i>	51,945 \pm 1.02	1,57,000 \pm 1.01	3,38,712 \pm 1.04	4,10,470 \pm 1.23

Table 2. CBC of normal control, *CpMT* and *CpAuNps* treated groups.

Parameters	Groups	Day 1	Day 3	Day 5	Day 7
CBC (cells/ μ L)	Normal Control	3,000 \pm 0.17	3,789 \pm 0.19	7,415 \pm 0.18	11,260 \pm 0.28
	<i>CpMT</i>	3,890 \pm 0.16	4,230 \pm 0.13	8,500 \pm 0.15	9,360 \pm 0.38
	<i>CpAuNps</i>	3,279 \pm 0.78	4,653 \pm 0.67	4,735 \pm 0.35	5,946 \pm 0.78

The observed enhancement in platelets and CBC may be assigned to the high surface area to volume ratio (nano sizing), biocompatibility, astonishing optical properties related to surface plasmon resonance and coating of medicinally important *Carica papaya* secondary metabolites (polyphenolic flavonoids) on freshly prepared nanoparticles. Overall, phenomenon allows accumulation and penetration of nano *CpAuNps* into living tissues comparatively deeper.

Phytochemical investigation on *Carica papaya* has reported the presence of various glycosidic flavonoids and terpenoids [22]. Polyphenolics like flavonoids and related compounds have been reported to enhance platelet and CBC in the patients of dengue fever [23,24]. It is reported that the extract of papaya enhances the gene expression of arachidonate 12-lipoxygenase or platelet-type lipoxygenase (*ALOX12*) and platelet-activating factor receptor gene (*PTAFR*) many folds finally promoting platelet production via the increased number of megakaryocytes [25].

CONCLUSION

The present study explores the role of green nanotechnological enhancement in the bio-efficacy of any homeopathic mother tincture. Gold nanoparticles are bio-fabricated using homeopathic *Carica papaya* mother tincture in a single step green method. After thorough characterization of bio-fabricated gold nanoparticles embedded with biomolecules of *Carica papaya* tincture (*CpAuNps*) was monitored for enhancement in platelet counts and CBC of patients suffering from dengue fever. The statistically significant enhancement in platelet and CBC of the patients was observed. The action of bio-fabricated gold nanoparticles (*CpAuNps*) at the nuclear level on the gene expression of arachidonate 12-lipoxygenase or platelet-type lipoxygenase (*ALOX12*) and platelet-activating factor receptor gene (*PTAFR*) is under study.

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