Folate receptor targeted naringenin gold nanoparticles exhibit excellent *in vitro* antioxidant potential

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Abstract

The present investigation revealed that the bio molecules present in citric phyto-compound naringenin are responsible for the synthesis of AuNPs and modified by folate (NAR-AuNPs-F). Characterizations of newly synthesized targeted nanoparticles were confirmed by Dynamic Light Scattering (DLS) study and Fourier Transform Infrared (FTIR) spectroscopy. In assessing the chemo-preventive nano drugs, their antioxidant properties are broadly appraised. From this perspective the antioxidant activity of biogenic folate modified naringenin gold nanoparticles (NAR-AuNPs-F) was assessed. I*n vitro* antioxidant activity was evaluated through the measuring 2,2- diphenyl-1-picrylhydrazyl, nitric oxide, superoxide, and lipid peroxidation scavenging activities. DLS measurement confirmed the nanoscale size of freshly prepared NAR-AuNPs-F that was 148 nm. FTIR analysis showed the presence of various functional groups i.e. hydroxyl, C=N which were responsible for the reduction and capping of AuNPs. It was found that nitric oxide, DPPH, lipid peroxidation, superoxide anion, scavenging actions of NAR-AuNPs-F treated groups were nearly similar to the standard antioxidant, ascorbic acid. The findings confirm1 that NAR-AuNPs-F may be an effective phytochemical based antioxidants agent.

1. Introduction

The development of biologically synthesized nanoparticles is a budding branch of nanotechnology and this synthesis of nanoparticles is also eco-friendly and encouraging approach in the arena of nanotechnology. Therapeutic uses of gold nanoparticles (AuNPs) were proved safe and less toxic in drug delivery systems [1]. AuNPs have been used to target

various cancer cells by using folic acid (FA) as it is useful as a ligand to enhance AuNPs endocytosis by folate receptor targeting different cancer and normal cell membranes. Synthesis of nanoparticle using various phytochemicals by eco-friendly green synthesis methods is safe for biological uses and less costly. Naringenin , a flavanone found in many citrus fruits, possess anti-inflammatory, antioxidant, and free radical scavenger properties [2]
Oxidative stress is onsidered as an oxidative imbalance for the inability to scavange the reactive oxygen species (ROS) during cellular metabolism [3]. The elevation in free radicals produced from biological oxidation causes disruption in intracellular proteins and leads to membrane damage due to peroxidation of membrane lipids. ROS also results in chromosomal changes by DNA strand breaks as well as by forming DNA and protein cross-links. Consequently cell death occurs by oxidative damage of cellular components [4]. Oxidative stress causes elevation in ROS which are related to the pathophysiology of various diseases [5]. Antioxidants safeguard any organism from the deleterious effects of free radicals by diminishing or repairing the damage. The biogenic nanoparticles can be utilized against detrimental effects of free radicals and may be competent antioxidant [6].
The focus of this paper is to synthesize and characterize folate modified naringenin gold nanoparticles and evaluate the antioxidant properties of the nanoparticles.

2. Materials and methods

2.1. Chemicals and reagents

Naringenin was purchased from Sigma Aldrich. Analytical grade hydro-chloroauric acid (HAuCl4), sodium borohydrade (NaHB4), tri sodium citrate, and potassium bromide (KBr) were procured from Merck India, Ltd., Mumbai, India. Ascorbic acid, hydrogen peroxide, methanol, phenazinemethosulfate (PMS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), and other chemicals were purchased from SRL chemicals, India .

2.2. Synthesis of folate receptor targeted biogenic gold nanoparticles using Naringenin

At first, naringenin (0.002mg/ml) was suspended in 50 ml millipore water. Gold nanoparticles of NAR (NAR-AuNPs) were synthesized by treating hydrochloroauric acid (5mM) in 50 ml solution of naringenin at room temperature for 30-45 min and were air dried. Air dried pellet were dispersed in 25 mM 2-mercaptaethanol in 30 ml ethanol and kept for 1 h 37ºC. The mixture solution was then centrifuged at 12,000 rpm followed by washing thrice.[7]
The precipitate was resuspended in 100 ml aqueous solution of mixture of folic acid (FA) and N-(3-dimethyl –aminopropyl N- ethylcarbodimide hydrochloride (EDAC). The ratio of FA to EDAC is 10:1 (mg) at pH 5. The solution was continuously stirred at 37ºC for 5h at 600 rpm. Then to stop the chemical reaction pH of the solution was checked to 9. Thereafter, the precipitate was collected by centrifugation at 13,000 rpm and air dried [8]

2.3 Characterization of NAR-AuNPs-F

2.3.1 Dynamic light scattering (DLS)

Zetasizer Nano ZS instrument (Malvern Instruments, U.K.) was used to measure dynamic light scattering (DLS) of NAR-AuNPs-F at 25 ± 1 °C constant temperature.
For DLS the nanoparticle solutions were syringe-filtered (0.2μm). The measurement of NAR-AuNPs-F was done thrice. The equilibration time period was fixed at 60 s. The polydispersity

index (PDI) was also determined for the detection of the particle size distribution. In both cases the pH of NAR-AuNPs-F were fixed [8]

2.3.3 Fourier transforms infrared spectroscopy (FTIR)

FTIR analysis of the dried NAR-AuNPs-F sample was performed by FTIR spectrometer (Perkin Elmer Spectrum Express-1.03.00) to detect the presence of different biomolecules. Data was recorded between 4000 cm^{-1} and 400 cm^{-1} wavelength.

Both NAR-AuNPs-F and naringenin were recorded at 4cm^{-1} and accuracy of ±0.01cm^{-1} .The spectra of different samples were examined. Potassium bromate (KBr) pellet was used as reference spectrum for the accuracy [7,8]

2.4. *In-vitro* antioxidant activity

2.4.1 DPPH scavenging assay

At first, 2.8 ml of naringenin (NAR), naringenin gold nanoparticles (NAR-AuNPs), Folate modified naringenin gold nanoparticles (NAR-AuNPs-F) were mixed with 0.2 ml (100 µM in methanol) of DPPH and ascorbic acid (10-200 µg/ml in methanol; used as standard drug) at 50, 100, 150, 200 µg/ml. Then 3 ml of this reaction mixture was incubated for 30 min at 37ºC in dark condition. Optical density was quantified spectrometrically at 517 nm (ShimadzuU-245spectrophotometer). The percentage inhibition was determined by the following formula [9].

$$\textbf{Percentage inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Absorbance of the control and T = Absorbance of the test sample.

2.4.2 Nitric oxide scavenging activity

Sodium nitroprusside (1ml of 10 mM), NAR, NAR-AuNPs, NAR-AuNPs-F (1 ml of each) at the above said concentrations and ascorbic acid at pH 7.4 phosphate buffer were thoroughly mixed and incubation was made for 2.5 h at 25°C. 1 ml of Griess reagent then was added in 1 ml of this incubated mixture. Absorbance was noted at 546 nm and the percentage inhibition was determined by the following way [10].

$$\textbf{Percentage inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Absorbance of the control and T = Absorbance of the test sample.

2.4.3 Superoxide anion scavenging assay

At the above said different concentrations 3 ml of NAR, NAR-AuNPs, and NAR-AuNPs-F were mixed with nitrobluetetrazolium (1 ml of NBT), and NADH (1 ml). Then after addition of 100 µl of phenazine methosulfate and incubation was made at 25ºC for 5 min and absorbance was noted at 560 nm to calculate the percentage inhibition by the above said formula [11]

2.4.4. Lipid peroxidation scavenging assay

To produce a 1/10 homogenate, adult male rat liver was homogenized with an ice-cold Tris-HCl buffer (20mM, pH -7.4). After centrifugation at 12,000 rpm (at 4°C) for 15 min, the supernatant was used for *in vitro* lipid peroxidation study. In each tube at previous concentrations of NAR, NAR-AuNPs, NAR-AuNPs-F, 1 ml of supernatant, 30mM KCl, $FeSO_4$ (0.16 mM), 0.06 mM ascorbic acid were mixed and incubation was done for 1 h at 37°C. Then 1.0 ml of TCA (10% w/v) and 1.5 ml of TBA (1% w/v) were mixed to make the

final volume of 4 ml by adding distilled water and keeping in water bath was accomplished for 30 min. Reaction mixture was made cooland . Then adding distilled water (1 ml) and 5 ml of n-butane: pyridine (15:1v/v) it was centrifuged for 10 min at 4000 rpm. At 532 nm organic layer of colored malondialdehyde (MDA)-TBA complex was measured [12]. The percentage inhibition was calculated by the same way.

2.5. Statistical analysis

Results were tabulated as Mean ± SEM. Statistics was done using one way ANOVA. Significance were considered at $p<0.05$ level.

3 Results:

3.1. Characterization

3.1.1. Dynamic light scattering and zeta potential measurement

From dynamic light scattering (DLS) the mean size of NAR-AuNPs and NAR-AuNPs-F (in aqueous solution) was measured. The average size (z-average) of nanoparticles from DLS measurements (Figure 1 A) was 23.66 nm and 148 nm (Figure 1 B). A change in colour, from monochrome to violet was observed while mixing naringenin into an aqueous hydrochloroauric acid solution. Further, the color of the solution changed when conjugation of folic acid took place. The pallet color changed to white.

3.1.2. FTIR analysis

FTIR analysis results of both NAR-AuNPs and NAR-AuNPs-F (figure 2.B and 2.C) examined the biomolecules responsible for capping as well as stabilizing the synthesized nanoparticles. The peaks of NAR-AuNPs and NAR-AuNPs-F are detected at 3440 cm-1 belongs to mainly the stretching of OH group. The shifts in the peak positions from 3440 to 3460 and 3470 cm-1 respectively. The shifts in the peak positions from 1637 to 1627 and then again 1637 cm-1 respectively for C=N bending. It indicates the involvement of naringenin in reducing as well as stabilizing the synthesized folate conjugated gold nanoparticles.

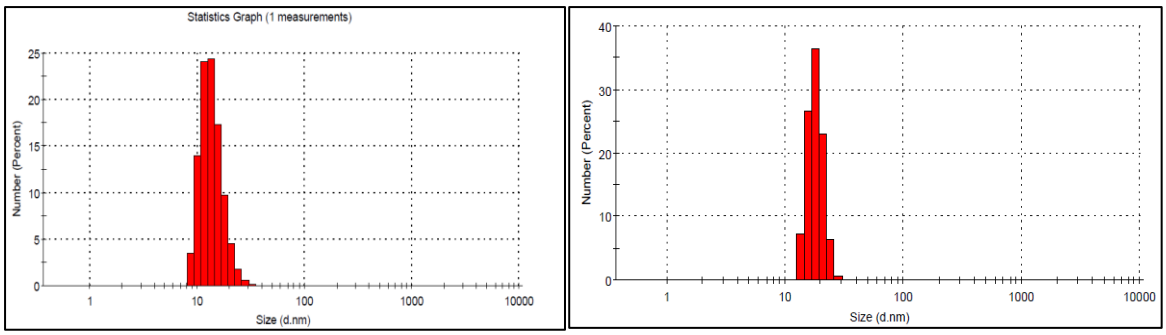

Figure 1 A and B: Size distribution of synthesized gold particles (NAR-AuNPs) and folate conjugated gold particles (NAR-AuNPs-F)

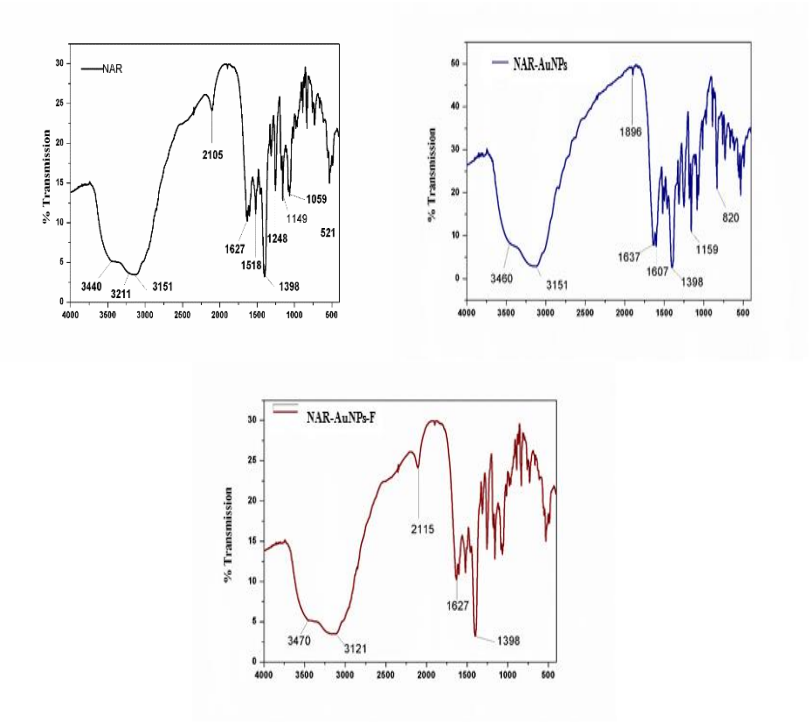

Figure 2. A, B and C: FTIR study of naringenin (NAR),Naringenin synthesized gold particles (NAR-AuNPs)andFolate modified naringenin synthesized gold particles (NAR-AuNPs-F)

3.2. *In-vitro* antioxidant activity of NAR, NAR-AuNPs, NAR-AuNPs-F

NAR, NAR-AuNPs, NAR-AuNPs-F showed inhibition on DPPH radical scavenging in dose dependent manner (Figure 3) and the IC_{50} value of NAR-AuNPs-F was 86.29 µgml^{-1}

In Figure 4 NAR-AuNPs-F exhibited nitric oxide scavenging activity. The respective IC_{50} values of NAR-AuNPs-F and ascorbic acid were 65.54 and 70.79 µgml^{-1}.

From the figure 5, it is shown that NAR-AuNPs-F has noticeable superoxide anion scavenging activity. The respective IC_{50} values of NAR-AuNPs-F and ascorbic acid were 85.81 and 86.24 µgml^{-1}.

The IC_{50} values of lipid peroxidation scavenging activity (Figure 6) of NAR-AuNPs-F and ascorbic acid were found to be 86.23 and 90.8 ugml^{-1} respectively.

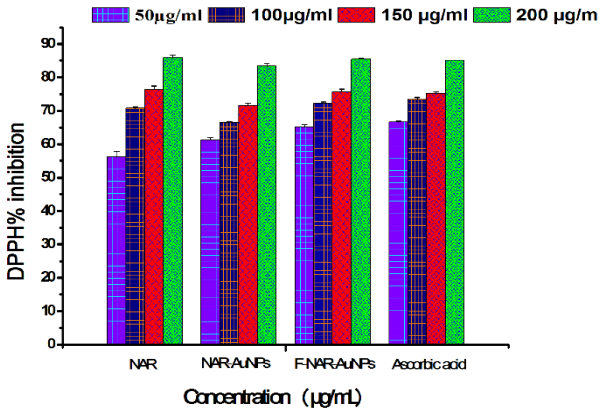

Figure 3: Effect of NAR, NAR-AuNPs, NAR-AuNPs-F and ascorbic acid on Diphenyl-2-Picrylhydrazine (DPPH) scavenging activity. Results are described as Mean ±SEM.

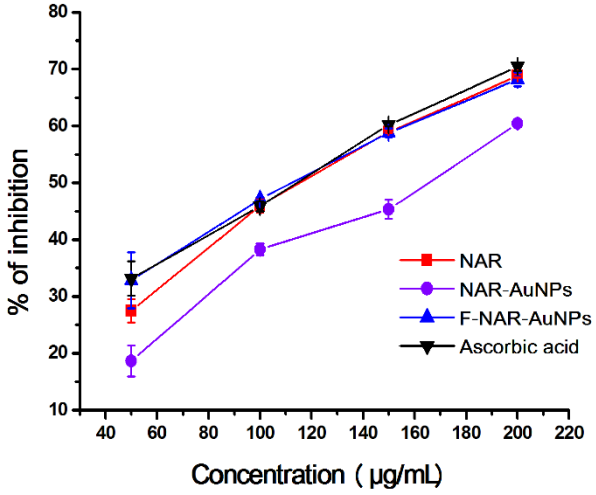

Figure 4: Nitric oxide scavenging activity of NAR, NAR-AuNPs, NAR-AuNPs-F. Values are stated as Mean ±SEM.

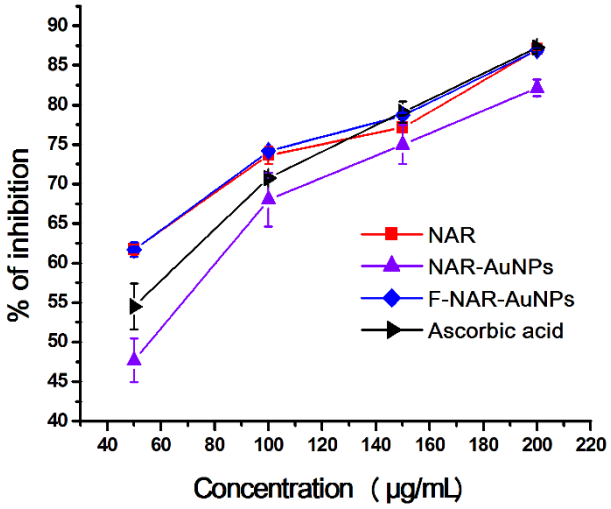

Figure 5: Superoxide anion scavenging capability of NAR-AuNPs-F and ascorbic acid Results are described as Mean ±SEM.

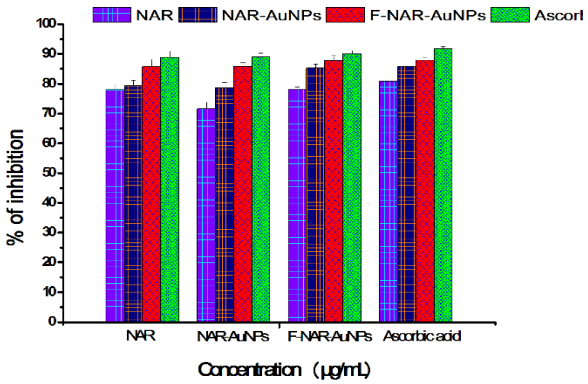

Figure 6: Effect of NAR, NAR-AuNPs, NAR-AuNPs-F on Lipid peroxidation scavenging activity. Values are stated as Mean ±SEM.

4. Discussion

This study describes the reducing characteristics of gold ions into folate receptor targeted nanoparticles containing naringenin and ultimately the targated synthesis of NAR-AuNPs-F. Distribution of the synthesized NAR-AuNPs-F size was confirmed by DLS technique (Figure 1). Size distribution histogram of particles revealed polydispersed natures both the NAR-AuNPs and NAR-AuNPs-F. The hydrodynamic average sizes (z-average) of two above mentioned nanoparticles found from DLS (Figure 1A and B) were respectively 23.66 nm and 148 nm.

FTIR was used to confirm the presence of possible biomolecules accountable for the gold ions reduction and capping of the synthesized gold nanoparticles. The FTIR spectra of NAR, NAR-AuNPsand synthesized NAR-AuNPs-Fare shown in (Figure 2).The peak at 3440 cm^{-1} belongs to mainly the stretching of OH group. The shifts in the peak positions from 3440 to 3460 and 3470 cm^{-1} respectively. The peaks positioning between 1637 and 1627 is due to C=N bending. It indicates the involvement of naringenin in reducing as well as stabilizing the synthesized folate conjugated gold nanoparticles.

The antioxidant potential of the nanoparticle NAR-AuNPs-F was established in the present study. A (free-radical scavengers) antioxidant, act on DPPH to yield DPPHH having less amount of hydrogen and show lower OD than DPPH. DPPH solutions after combining with the hydrogen atom source, form the lower state of diphenylpicrylhydrazine yielding its violet color [13]. NAR-AuNPs-F as well as ascorbic acid augmented the DPPH scavenging activities in dose dependent manner (Figure 3).

To prevent the incidence of inflammation, fast clearance of reactive nitrogen species (RNS) by antioxidants is vital. Plant polyphenols are commendable scavengers for RNS. Numerous plant-based flavonoids are reported to be modulators for several diseases, such as hepatitis virus, SARS-CoV-2, autoimmune disease, type 2 diabetes, cardiovascular diseases, and cancer etc [14]. Excessive formation of NO is related to a number of diseases. In this present study, NAR-AuNPs-F exhibited effective nitric oxide scavenging action in a dose-dependent manner (Figure 4). It may be due to its capability to inhibit the formation of nitrite molecules which can directly interact with oxygen and nitrogen oxides [15].

Biomolecules are directly or indirectly damaged superoxide anions by creating singlet oxygen or hydrogen peroxide in aging and other pathological processes and then, lipid peroxidation becomes to initiate [16]. Figure 5 represents the superoxide radical scavenging effect of NAR-AuNPs-F nanoparticle, and ascorbic acid in a dose-dependent manner.

Various pathological events are associated to lipid peroxidation of cellular membrane [17] . It was reported that many terpenoid compounds inhibit lipid peroxidation [18]. From the present study it was seen that NAR-AuNPs-F acted as a significant antioxidant which could exhibit different ROS scavenging activity Figure 6.

5. Conclusion:

NAR-AuNPs-F showed potent *in vitro* antioxidant activity prospective and from the study it is revealed that biogenic NAR-AuNPs-F may be a promising candidate in regulating oxidative stress. NAR-AuNPs-F exhibited more potent antioxidant activity than that of NAR and NAR-AuNPs.

6. Conflict of interest

The authors declare that they have no known conflict of interest.

7. Acknowledgements

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