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RESEARCH ARTICLE

In vivo study of gold-nanoparticles using different extracts for kidney, liver function and photocatalytic application

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Abstract: We report novel gold nanoparticles by green method for different fruit extracts have gaining greater attention due to its versatile properties in different applications. In this article, GNPs synthesis is demonstrated successfully using fresh fruit extract of *punica granatum* and *fragaria*. The optical properties, morphology and elemental analysis of samples was done by using) different characterization techniques like SEM EDX and UV-Visible spectroscopy Biocompatibility of GNPs was determined by ALT, AST, ALP, Urea, Creatinine tests and also to investigate the effects of prepared GNPs on the kidney and liver functions. The GNPs prepared by fruit extract of punica granatum have more effects on the rabbits than GNPs prepared from fragaria but this effect normalizes after three days which shows its biocompatibility. To explore the photocatalytic activity of the GNPs the photocatalytic degradation of MB dye is also investigated. The results revealed that GNPs prepared through green synthesis route are found to be efficient enhancement in the degradation of MB dye in visible region due to large surface area. These particles were more active in catalytic reduction due to their high surface energy and in bio-medical applications as biocompatibility.

Keywords: green method, AuNPs, biocompatibility, photocatalytic activity

1 Introduction

Nanoparticles act as a bridge between bulk materials and atomic or molecular structures as they have variety of scientific interest. Now a days, evolution in material sciences are experiencing broad field work by interacting with different scientific disciplines and making strong effect on every fields of life.[1] The Au NPs have discovered their tracks in the disciplines of life sciences or biological sciences in view of their biocompatibility and reaction given by the human body it is because of golds non-poisonous nature and inert core. The color of gold nanoparticles is red because of its property of the surface Plasmon resonance (SPR).[2,3] Gold nanoparticles have high absorption cross sectional area, High solubility, Productive SPR has simple linkage with targeting particles and drug due to its peaks at longer wavelength. These characteristics make GNPs a promising member for cancer thermal treatment and different pathogenic sicknesses.[4] A gold nanoparticle as a result of its optical, physical (Quantifiable) and chemical importance was considerably more useable in the area of life sciences. The inert character of the Au NPs is additionally valuable for the determination of cancer cells, impetuses, utilized in the drug delivery and so forth.[5] Au NPs has extensive vivo biomedical applications (BME) particularly as a malignant cell growth , imaging as well as in treatment.[6] The Au NPs effectively works with targeting biomolecules via thiol-gold conjugation method.[7,8] Gold nanoparticles are used to design conductors from printable inks which print on the electronic chips as used in computers to make their speed fast.[9] In medical, the gold nanoparticles can be used for the diagnosis of heart diseases, infection, tumor and cancers; to detect biomarkers.[10] There reported many strategies for the preparation of Au NPs like reduction method,[11] sol-gel technique,[12] micro emulsion approach,[13] hydrothermal technique,[14] green approach,[15] and so on and numerous different strategies utilized op-down approach resemble sono-chemical

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technique, vapor deposition by chemicals, electros pinning, electrophoretic deposition, heating stirring method ,micro-emulsion method, laser ablation, and mechanical milling, *etc*. The green synthesis is another viable technique for the preparation of gold nanoparticles. Malva crispa (mallow) utilized for the amalgamation of Au NPs along with strong antibacterial or antimicrobial agent against a bacterium, virus, or other microorganism and sustenance decay.[16] The Au NPs in range 20-30 nm are reported to be extracted by using aqueous leaves of Acalypha indica.[17] Triangular shaped gold nanoparticles were also prevail through Au ions reduction by the fluid of lemongrass.[18] A reducing and capping agent such as *Cymbopogon citratus* leaf extract is also reported for the synthesis of gold nanoparticles.[19] Biosynthesis of *Zingiber officinale* leaf extract to form 10 nm gold nanoparticle size is also reported earlier.[20] The synthesized nanoparticleshas great capacity to produce the drugs and used against fungal diseases.[21] A solid gold nanoparticle with changing size variable size was procure obtained by with using the extract of the *endophytic fungus* leave synthesis.[22] The *Punica granatum* juice used for the preparation of AuNPs for the application in a cancer targeted drug delivery.[23–25] Quercetin was packed within disulfide-modified mesoporous silica together with GNPs (Q-Au/SiO2) which had voids around GNPs and final materials can refine its packing and efficiency in drug carier.[26] The thermoresponsive hyperbranched polymer functionalized with GNPs synthesize through in-situ chemical reduction method is reported earlier in which, the green preparation method is used with further applications in unusual colorimetric sensor along with energy saving, environmental protection, and sustainable development features.[27] A basic in-situ preparation method was developed to manufacture, complex of Tremella fuciformis (TF) and gold nanoparticles (AuNPs). The intensities of the localized surface plasmon resonance (LSPR) of the complex of TF and GNPs increased due to drying.[28] In Previous research work an substitute method used for making extremely stable GNPs, where an inert Curcuma mangga (CM) which act as stabilizing and reducing agent juice, was used to overcome the previously mentioned requirements. Blood tests were taken which divulge that prepared GNPs with less than 10% of hemolysis without any accumulation of erythrocytes were blood-compatible. The further research reveals that for the preparation of GNPs ,there is great possibilities by involving a CM-extract-based method for anticancer diagnosis and therapy.[29] From literature, Au NPs synthesized from *Punica granatum* was used as a cancer targeted drug delivery. In this study, we reported the same green method from *Punica granatum* with varying ratios. This work additionally reports the green synthesis of AuNPs at ambient temperature nearly equal to 20°C by utilizing natural product concentrate of Fragaria (strawberry) as reducing gent which is another option in writing. The previous results of GNPs from *Punica granatum* has been compared with synthesis of GNPs via using *Fragaria* (strawberry). Previous studies on biomedical applications resulted that GNPs insert into animal bodies get gather together or acquire an increasing number in the liver and kidneys, which could be dangerous. This work includes the animal studies through which GNPs biocompatibility was confirmed so this research work was further helpful for humans due to its biocompatibility.

2 Materials and Methods

The 250 g and 100 g edible grains of *Punica granatum* and *Fragaria* (Strawberry) were clean twice with normal tap water than once with DDW, then cut into small cubes in a blade bleender with 50 mL of DDW until a uniform mixture formed. The prepared solution of *Punica granatum* was centrifuged in order to wash the sample for 2 minutes, with next step of filtering through whatsman filter paper and stored at -18°C for further utilization. Precursor solution was prepared by using 30 wt% Chloroauric acids (HAuCl4) in 50 mL of DDW to form a 0.1 g/L solution. 0.75 mL of fruit extract of *Punica granatum* and *Fragaria* (Strawberry) was added in prepared precursor solution in order to synthesized gold nanoparticles. It was perceive that the color of solution transform into deep purple/red within few seconds under constant stirring at room temperature while slight heating (35°C-40°C) for several minutes were compulsory respectively. The color transformation as shown in figure 1 was due to presence of AuNP, as clarified through UV-vis absorption at 530 nm and 528 nm. (see Figure 1).

Figure 1. Preparation of GNPs (Color changes after 15mins to 30mins)

3 Results and Discussion

3.1 SEM analysis

The SEM examination was follow out with Hitachi S-4500 SEM. For imaging, carbon-coated grid utilize for thin film preparation of samples, coated with carbon were taken in a small amount needed on the grid and extra solution is remove by using paper. Then the film was dried by putting SEM grid under mercury lamp for five minutes and the picture was made. The GNPs has spherical like shape with an average diameter ranging from 25-35 nm as clearly shown in Figure 2(A), which were synthesized at room temperature *using Punica granatum* fruit extract. The SEM images in Figure 2(B) clearly indicates aggregation formation of spherical like GNPs with an average diameter ranging from 30-45nm as prepared by using *Fragaria*. The SEM images clearly illustrate that, prepared GNPs from fruit extract of *Fragaria* are more stabilized than *Punica granatum* due to containing rich primary and secondary metabolisms.

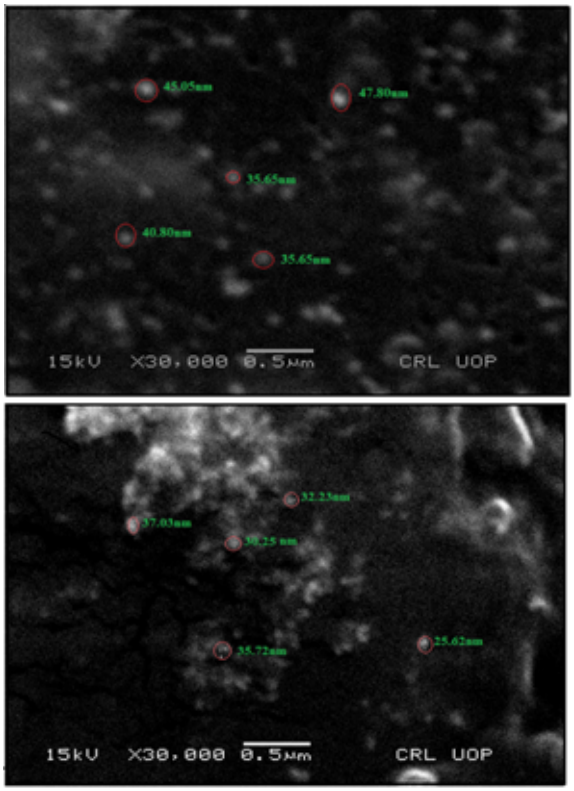

Figure 2. (A) SEM images for the GNPs from *Punica granatum*; (B) SEM images for the GNPs from *Fragaria*

3.2 EDX analysis

The EDX spectroscopy has carried out in order to identify elemental percentage in the GNPs samples. It depends upon the atomic mass of the element which is to be detected. So, if samples have mixed elements with wide range of atomic numbers then detector peals size will not vary with the compositional ratios. The EDX spectra of prepared GNPs using *Punica granatum* and *Fragaria* are appeared in Figure 3(A) and Figure 3(B), respectively. The EDX profile indicated highest signal for GNPs at KeV with some other adjoint elements such as Mg, K and Ca. The organic compounds (carbon and oxygen) are absorbed on the surface of GNPs from fruit extract of *Punica granatum* and *Fragaria,* which plays a great role in the reduction and capping of GNPs. The EDX spectra illustrate that, *Fragaria* have rich capping and reducing capability than *Punica granatum.*

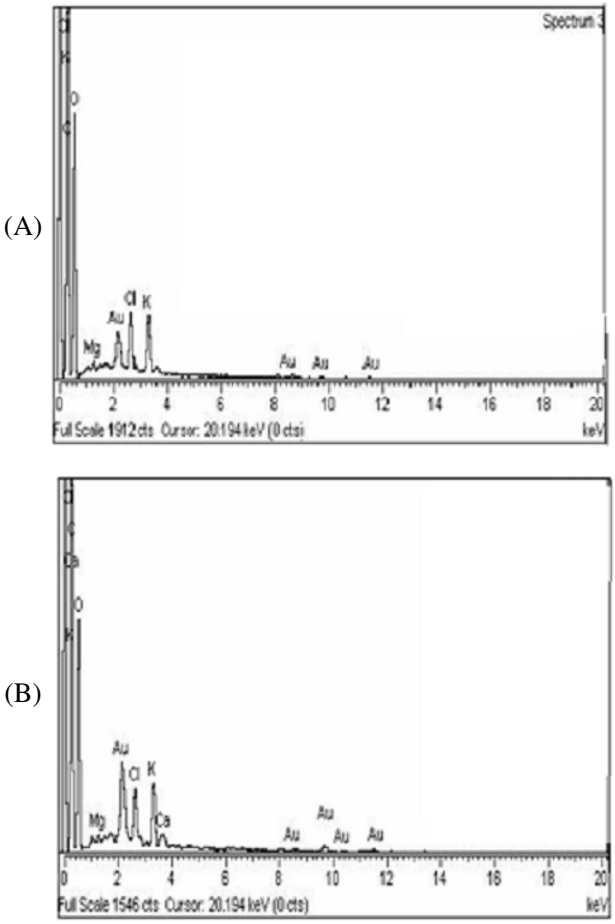

Figure 3. EDX profile of gold nanoparticles prepared from (A) *Punica granatum* (B) *Fragaria*

3.3 UV-Visible spectroscopy analysis

The optical properties and formation of GNPs were explored by using UV-Visible spectroscopy. The UV-Visible spectra for GNPs prepared by using fruit extract of *Punica granatum* and *Fragaria* respectively are shown in Figure 4(A) and Figure 4(B). The maximum

absorption peaks are observed at 528nm and 530 nm, respectively. These peaks clearly indicate thatthe prepared GNPs exhibits surface plasmon resonance phenomena at 528 nm and 530nm. It is worth mentioning that the absorption peak of GNPs prepared by using *Fragaria* are broad than prepared by using *Punica granatum.*

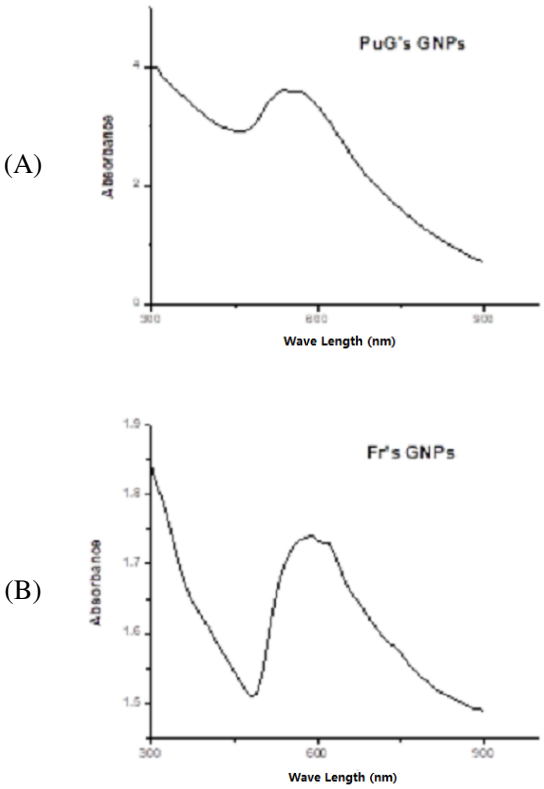

Figure 4. (A) shown UV-Visible spectra of GNPs prepared by using fruit extract of *Punica granatum* at 528nm (B) indicates UV-Visible spectra of GNPs by using fruit extract of *Fragaria*

3.3.1 Animal studies

As indicated by the guidelines and methodology set by the University of Veterinary and Animal Sciences, Lahore (UVAS), the animal care was carried out. Two healthy albino rabbits were chosen. GNPs prepared by *Punica granatum* administrated by one of the rabbits and second one rabbit with GNPs prepared by Fragaria. For this 0.8 mg of gold nanoparticles were scattered in 0.8 ml saline buffer solution. So two injections were prepared, first GNPs prepared from *Punica granatum* sample and second from Fragaria sample These injections were injected in male rabbits through marginal ear vein.

After the two and three days of injection, the blood tests were taken and were broke down for biochemical blood parameters (glucose and cholesterol levels), renal function (blood urea and serum Creatinine, liver function Alanine Transferase [ALT] Alkaline phosphatase [ALP] and aspartate aminotransferase [AST]).(See Figure 5)

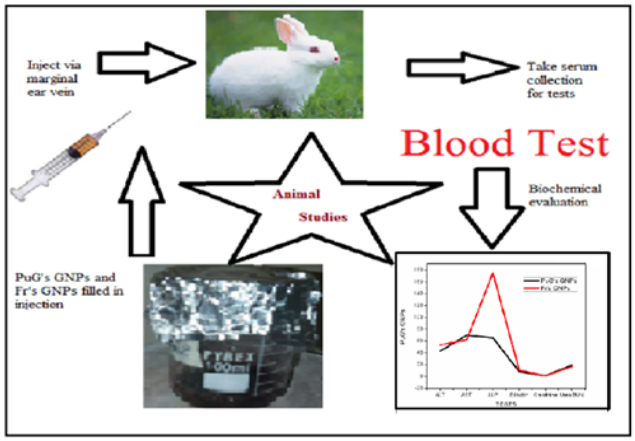

Figure 5. Process for animal studies in albino rabbits. PuG's GNPs means gold nanoparticles prepared from *Punica granatum* and Fr's GNPs from *Fragaria*

Priorly theexaminations about nanoparticles have demonstrated the AuNPs insert in the bodies of animals accumulated within liver and kidneys that would be lethal and results in the demise of animals. This work reported the biocompatibility of GNPs and its consequence on liver and kidney function have been studied in albino rabbits. All rabbits remain alive during the 3-day study period after injection, and no progressions were seen in typical conduct, for example, nourishment utilization and physical capacity.

After injecting prepared gold nanoparticles has shown a slight increase in ALT levels and AST level as in (Figure 6 (A,B)) which decreases slightly after the first Day injection in AST and on the other hand ALT decreases and went normal on third day after injection. For the next 3 days after injection, the statistic information was composed. ALT level at first 2 days tend to get normalize on 3rd day and have no fetal effects and liver destruction. AST and ALP found in the liver and other tissues is look over regularly according to the liver along ALT and bilirubin.

ALP level (Figure 7C) increases on first day and decreases on second and third day. Total bilirubin (Figure 7D) directly decreases after the first day injection then remains same on the second day. The level of total bilirubin endure constant for 2 days than expanded somewhat on the third day in the injected rabbit Fr. GNP. Here, bilirubin is a component of red blood cells, including the liver and ALT is an enzyme that is present just in living.

Renal function is evaluated by blood urea (Figure 8E) and creatinine (Figure 8F) by the kidneys. Our outcomes demonstrated a little bit decreasing of creatinine level

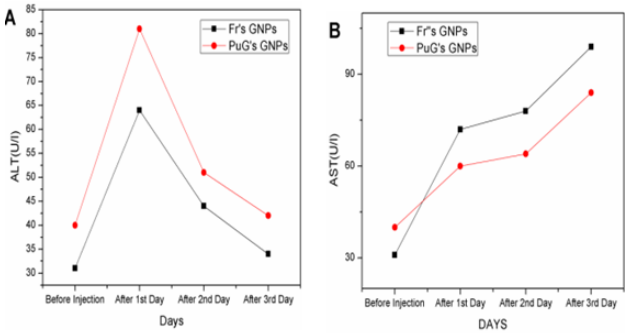

Figure 6. Effect of GNPs on ALT and AST level

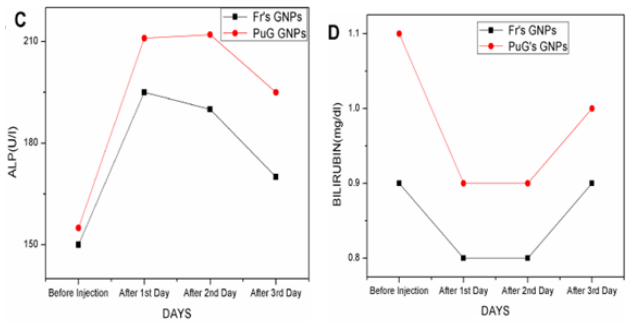

Figure 7. Effects of GNPs on ALP and Bilirubin level

(Figure 8F) following third days of Fragaria GNPsBy result of liver and kidney function, it is noteworthy that the renal function is more influenced by the GNPs of Punica granatum compared to the GNPs of Fragaria. However, in this work, it is obviously seen that both liver and kidney functions are influenced possibly after the two sorts of gold nanoparticles as reported.[30] The present study showed that contrast efficiency of both GNPs from Punica granatum and GNPs from fragaria is in agreement with previous studies.[31–33]

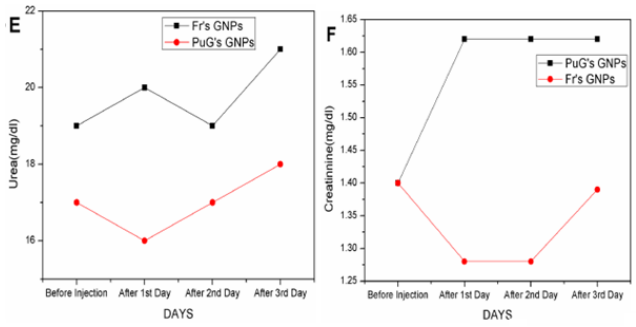

Figure 8. Effects of GNPs on Urea level and Creatinine level

3.4 Photocatalytic Degradation

Zeolite mixtures with gold nanoparticles (AuNPs/zeolite) have been synthesize prior by advancement of zeolite from coal fly fiery remains, strong waste poison, and its transformation to visible light active photoocatalyst via immobilization of GNPs by both in situ and ex situ methods.[34] The 5ppm aqueous solution of methylene blue dye was prepared at room temperature with prepared GNPs (1 g/L) as photocatalyst. It indicates the degradation of MB dye molecules with GNPs photocatalyst was analyzed as function of time. It is observed that, degradation rate was drastically increased with increasing time as well as maximum dye was degraded in 150 mintues of reaction time due to rich availability of active sites (Figure 9). The smaller particles exhibits maximum photo catalytic activity because of increasing surface to volume ratio with maximum number of active sites.

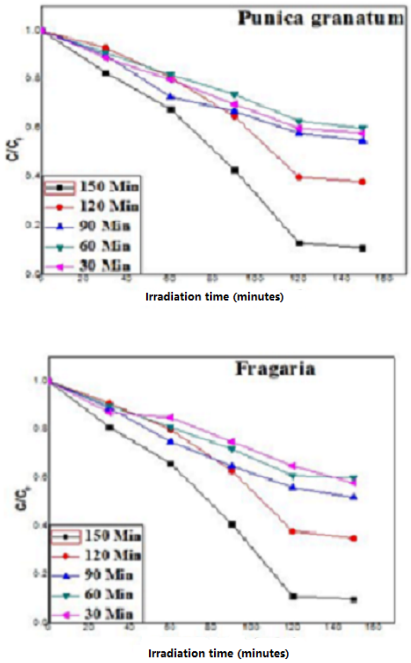

Figure 9. Degradation rate for the activity measurement with GNPs (1 g/L) photocatalyst using different extracts

4 Conclusion

The green synthesis technique is financially savvy, ecofriendly and can create GNPs at room temperature. *Punica granatum* and *Fragaria* fruit extracts have been used for the development of GNPs with spherical shapes. The average size of GNPs crystal by *Punica granatum* was found to be between 25 nm and 35 nm and that for GNPs prepared from Fragaria was of the order of 30 nm to 45 nm. This examination demonstrates the impacts of prepared AuNPs tests on liver and kidney capacities when rabbits were injected intravenously with

0.80 mg/kg body weight per dose of GNPs prepared by *Punica granatum* and *Fragaria* respectively. The impacts of gold nanoparticles on the biochemical parameters were assessed following 1, 2 and 3 days of intravenous (IV) injections, including the profile of liver function and renal (kidney) work. The final products indicates GNPs prepared by *Punica granatum* have more impact on the rabbits as contrast with Fragaria as there was some increase in the level of aspartate amino transferees (AST), antacid phosphate (ALP), and Serum creatinine however that impact normalize following days which demonstrates their biocompatibility. It was observed that the photocatalytic activity of the GNPs could be affected by increasing the number of active sites due to larger surface to volume ratio.

5 Conflict of interest

It is declared that the authors have no conflict of interest.

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