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RESEARCH ARTICLE

EFFECT OF DIETARY DUPPLEMENTATION OF GOLD NANOPARTICLE ON HISTOLOGY OF *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT

Research activities in application in application of nanoparticle on fish is increasing significantly. Areas for commercial development are the inclusion of nanoscale ingredients and use of nanotechnology in fish feeds, food packaging, the use of nano materials in the construction of aquaculture systems and application in fish health. Histology and histopathological alterations are effective biomarkers reflecting impact of environmental stressors, revealing prior alterations in physiological and/or biochemical function, culminating in impaired health (Handy *et al*., 2008). Biogenic gold nanoparticle have been used in the present study to evaluate the impact of its oral supplementation in *Oreochromis mossambicus.* These gold nanoparticle were prepared using 0.3 M $HAucl_4 3H_2 0$ (Sigma-Aldrich) and aqueous extract of *Ocimum sanctum and Curcuma longa*. Synthesis of biogenic and synthetic gold nanoparticle was confirmed from the UV-Vis study of surface plasmon resonance property of the colloidal solution. Juveniles of *Oreochromis mossambicus* in the range 7 ± 0.35 cm and 5 ±0.62 gm were stocked at 20 fish/1000L tanks and maintained at laboratory conditions. Experimental diet was prepared by incorporating 10ml of biogenic and synthetic gold nano solution per 100gms of basal feed. Non-treated control diets and aquous extract of *Ocimum sanctum* and *Curcuma longa* extract incorporated diets too were prepared. The experimental schedule was for six weeks and the fishes were fed at 2% of body weight twice daily. The biological effect was assessed in terms of histopathological studies at liver. The histopathological data revealed significant variations between treated and untreated groups. The details have been documented and discussed in the light of available literature.

Key words: Resource process, Resource base, Socio-economic transformation, Basalt Quarrying.

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INTRODUCTION

In recent years, nanotechnology is emerging as cutting edge technology interdisciplinary with physics, chemistry, biology, material science and medicine. The prefix nano is derived from Greek word nanos meaning "dwarf" that refers to things of one-billionth in size. The primary concept of nanotechnology was presented by Richard Feynman in a lecture entitled "There's plenty of room at the bottom" at the American Institute of Technology in 1959. Nanoparticles are usually 0.1 to 1000 nm in each spatial dimension. As interest in the potential benefits of nanomaterials have increased, the potential toxic effects resulting from use or unintentional release into the environment are concerned (Moore, 2006). Much of the toxicological research to date focus on atmospheric or inhalation exposures (Chen *et al.,* 2006); however, use of nanomaterials are likely to result in releases into aquatic systems and may pose a risk to aquatic ecosystems (Moore, 2006).

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Recently, significant concerns have been expressed about the potential risk of metal nanoparticles, due to the current and projected high exposure. And their likely high hazard and toxicity in the environment. Recent biological synthesis of metal nanostructures have gained tremendous popularity due to the environmentally friendly green chemistry approach. Nanoparticles are commonly synthesized using two strategies: top down and bottom up methods (Fendler, 1998). In top-down approach, the bulk materials are gradually broken down to nanosized materials whereas in bottom-up approach, atoms or molecules are assembled to molecular structures in nanometer range. Bottom-up approach is commonly used for chemical and biological synthesis of nanoparticles. Some toxic chemicals and hydrophobic solvents have the ability to reduce and stabilize the nanoparticles. These chemically synthesized nanoparticles carry some reactive functional groups, which can be toxic to biological system. Hence, the development of clean, biocompatible, non-toxic and eco-friendly methods for the synthesis of nanoparticles deserves merit. Biological methods are regarded as safe, cost-effective, sustainable and environment friendly processes. Microbes and plants, are regarded as potent eco-friendly green nanofactories. Biological synthesis of nanoparticles has been emerged as a promising field of research as nanobiotechnology interconnecting biotechnology and nanotechnology. In the present study the plant extract selected for biological synthesis of gold nanoparticles are aquous extract of *Curcuma longa* and *Ocimum sanctum.* A growing number of nanotechnology applications utilize metallic components, many of which can be toxic to aquatic organisms. Fish is a suitable indicator for monitoring environmental pollution because they concentrate pollutants in their tissues directly from water and also through their diet, thus enabling the assessment of transfer of pollutants through the trophic web (Fisk *et al*., 2001). Due to being exposed to pollutants, major structural damages may occur in their target organs, histological structure may change and physiological stress may occur. This stress causes some changes in the metabolic functions. The changes in the functions are initiated with the changes in the tissue and cellular level. According to Meyers and Hendricks, 1985,histology and histopathology can be used as biomonitoring tools or indicators of health in toxicity studies as they provide early warning signs of disease. Histopathological alterations are biomarkers of effect of exposure to environmental stressors, revealing prior alterations in physiological and/or biochemical function (Hinton *et al*., 1992). In the present study the sites selected for the histological studies is liver. The liver was examined because it plays a major role in the metabolism and excretion of xenobiotic compounds with morphological alterations occurring in some toxic conditions (Rocha and Monteiro, 1999).The liver of fish can be considered a target organ to pollutants and alterations in its structure can be significant in the evaluation of fish health(Myers et al;1998)and exhibit the effects of enviromental pollutants(Hinton *et al*., 1992). Currently nanoparticles are widely used,but their effects on cells are still under investigation.Reports dealing with long term effects of AuNPs on normal cells are less.Thus the interaction of nanoparticles with biological systems including living cells has become one of the most urgent areas of collaborative research in material science and biology.The present work is an attempt to find out the effect of gold nanoparticles synthesized through biogenic and synthetic method on the cytostructure of aquatic candidate *Oreochromis mossambicus.*

MATERIALS AND METHODS

Synthesis of gold nanoparticles: Ag 3+ to Au^{3+} Synthetic gold nanoparticles are prepared by the reduction of Auric chloride ($HAuCl_4$]), after addition of reducing agent(here sodium borohydride),the solution is rapidly stirred leads to the reduction of gold ion (Ag3+) to neutral gold atom(Au^0) and the continuation of the operation will turn out all the gold ions to neutral atoms and the solution becomes supersaturated. An addition of sodium borohydride saw an immediate change in the solution colour from yellow to purple (Fig: 1, 2, and 3). Tri sodium citrate is used as capping agent. The formation of colloidal gold nanoparticles was investigated using UV -Vis absorption. In the present study, green process for the production of gold nanoparticles uses direct interaction of $HAuCl_4$ with aquous extract of C*curcuma longa and Ocimum sanctum* in the absence of manmade chemical and thus satisfies all the principles of 100% green chemical process. Various phytochemicals present in the plant extracts presumably responsible for making a robust coating on gold nanoparticles and thus rendering stability against agglomeration. Absorption measure indicated that Plasmon resonance wavelength of synthetic gold nanoparticles and green synthesized gold nanosolutions from both the plant extracts is 520nm.Experimental diets were prepared by incorporating10 ml of the above mentioned concentrations of gold nanosolution per 100gms of basal feed prior to pressure pellatisation.

Fig. 1. Synthetic gold nanoparticle

Fig. 2. Biogenic gold prepared from *Curcuma longa*

Fig. 3. Biogenic gold from *Ocimum Sancta*

UV-Vis Spectrophotometric analysis of Gold Nanoparticle: Spectrophotometry is a important aspect of characterization of gold nanoparticles. With increase in particle size, the absorption peak shift to longer wavelength and the width of the absorption spectra is related to the size distribution range. Generally gold nanoparticles display a single absorption peak in the visible range between 510-550 nm because of the surface Plasmon resonance and show heavy absorption of visible light at 520 nm. This gives purple colour varies according to their size.

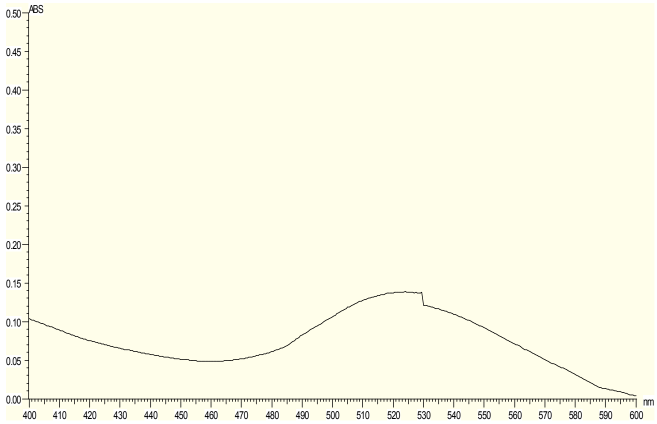

Fig. 4. UV-VIS Spectrum of Chemically reduced GNP

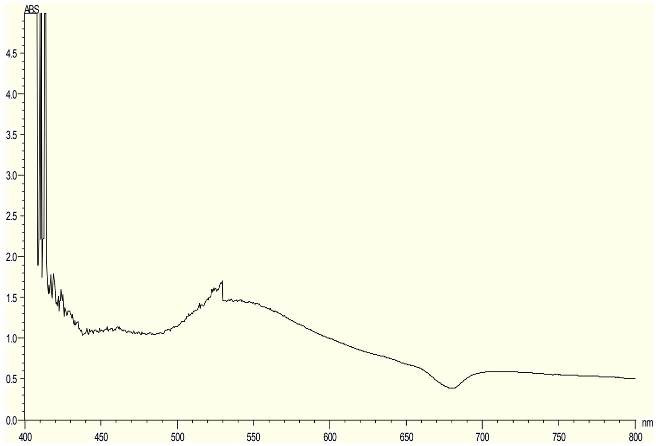

Fig. 5.UV -VIS Spectrum of nano *Ocimun sanctum*

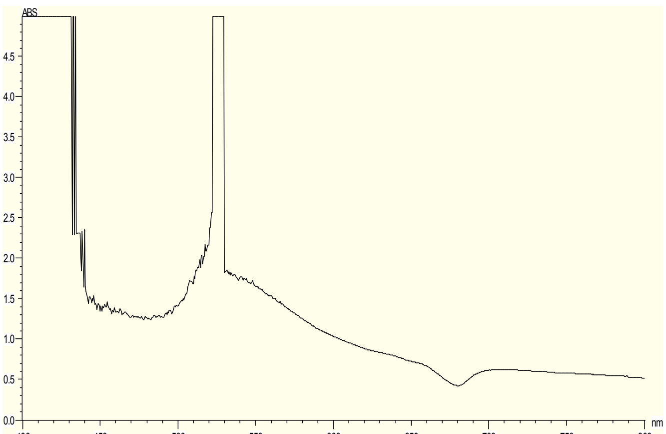

Fig.6. UV -VIS Spectrum of nanocurcuma

The appearance of violet colour is evident that the formation of gold nanoparticle in the reaction mixture and the efficient reduction of Au^{3+} to Au^{0} (Fig: 1, 2 and 3)). The formed colour solution allowed to measure absorbance against wavelength to confirm the formation of gold nanoparticle. The corresponding UV absorption spectra of the nano gold solutions in experiment is shown in (Fig: 4, 5 and 6). The absorption is a typical gold surface Plasmon vibration excitation for colloidal gold nanoparticles when they interact with electromagnetic radiation. In the optical absorption spectrum of the resultant nanoparticles the absorption wavelength of Gold nanoparticles were observed at 520 nm.. The absorption spectrum of both the green synthesized nanoparticles is shown in fig 5 and 6.In both the reaction mixtures the observed intensity of SPR peak is more with small sharpness in the peak suggesting complete reduction of gold nanoparticles. The reasonable narrow absorption peak indicated that the particles were not aggregated and the capping was effective.. Phytochemical constituents in the plants and spices extract like essential oils (terpenes eugenols etc), polyphenols and carbohydrate contain active functional groups,such as hydroxyl aldehyde and carboxyl units play important role for reduction of HAucl4 to AuNPs. Gold nanopartcles particles produced using phytochemicals or other extract components remain stable for prolonged period.

Experimental design: Sixty fishes belonging to both sexes and having an initial length of 7 ± 0.35 cm and 5 ±0.62 gm were were selected. The experimental setup consists of six tanks with 10 fishes per 10 litre of water. The fish in each set was fed with 2% body weight per day. First tank served as control.In the second and third tanks fishes were fed with diet containing gold nano solution prepared using aquous extract of *Curcuma longa* and *Ocimum sanctum* extract and the fourth and fifth tanks were fed with aquous extract of *Curcuma longa* and *Ocimum sanctum* incorporated diets.Sixth tank was maintained on chemically reduced gold nanoparticle incorporated diet. After 30 days of treatment, cytostructural profile of the selected tissue of different treatment groups were estimated.

Histological and histopathological studies: For histological and histopathological studies, small pieces of the liver and gill were taken from control and treated fish at the end of exposure regimen (30 days). Samples were fixed in 10% buffered formalin for twenty four hours at 4ºC, dehydrated in ascending grades of ethanol, immersed in xylol, and embedded in paraffin wax. Sections of 4-5 µm thick were mounted on clean glass slides, deparaffinized, rehydrated, stained with hematoxylin and eosin and mounted with DPX. Sections were examined using a light microscope (Roberts and Smail, 2001)

RESULTS

The accumulation of metals in aquatic organisms has been linked to decrease in survival and reduction of reproductive ability of aquatic organisms (Liao *et al.,* 2003). Toxicity of exposure to nanoparticles may vary with size, structure and composition of engineered nanoparticles (Fig. 6) (Griffitt *et al.,* 2008; Klaine *et al.,* 2008). Cytostructural studies of fish liver is an indicator of chemical toxicity and useful way to study the effects of exposure of aquatic animals to toxins present in the aquatic environment (Fernandes *et al.,* 2008).In fishes treated with synthetic gold nanoparticles, the most common lesions were vacuolar degeneration in the hepatocytes. Histopathological changes also observed, cloudy swelling of hepatocytes, hyalinisation, increased vacuolation associated with lipid accumulation, necrotic hepatocytes, hemorrhage, pyknosis, and cellular swelling. Vacuolisation of hepatocytes are associated with the inhibition of protein synthesis,energy depletion, disaggregation of microtubules or shift in the substrate utilization(Hinton and Laure'n,1990) Disturbances in protein synthesis causes hyalinization. Cellular swelling occurs either directly by degeneration of volume regulating ATPase or indirectly by disruption of the cellular energy transfer process required for ionic regulation (Hinton and Laure'n, 1990). According to Palaciose *et al.,* (2000), liver parenchyma showed signs of degeneration and focal necrosis on exposure to fishes to contaminants.

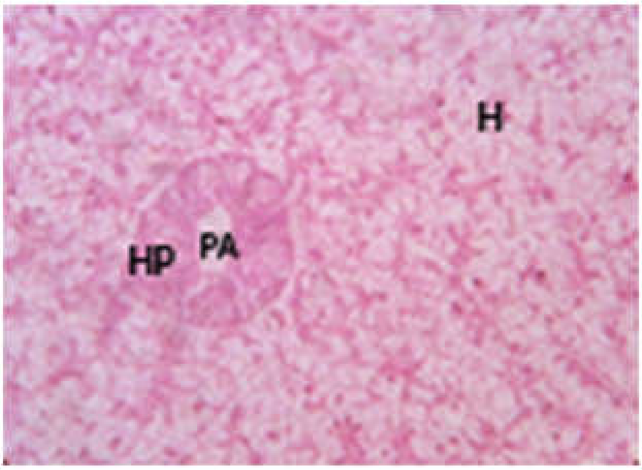

Fig. 7. Section of hepatopancreas of control *Oreochromis mossambicus* showing the hepatic part9(H) harbors normally organized hepatocyte with intact nuclei.the pancreatic portion (HP) possesses long elongated pancreatic cells proliferating around a portal artery(PA).40X.,

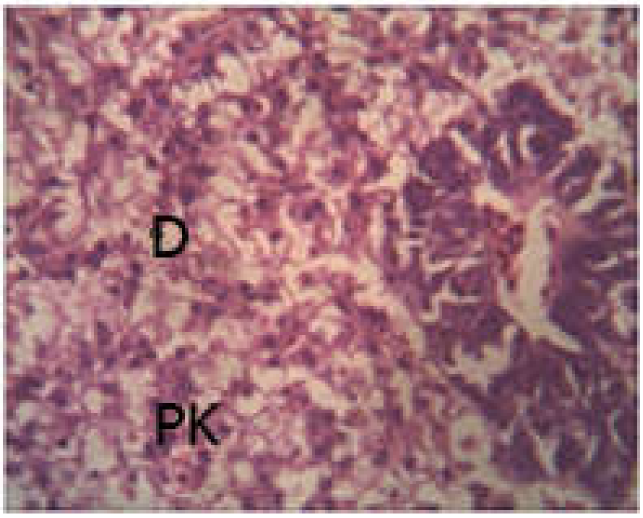

Fig. 8. Section of liver of *Oreochromis mossambicus* fed with Synthetic gold nanoparticle incorporated diet. In the hepatic portion, cytoplasmic degeneration(D) andv acuolar changes are demonstrated in the hepatocytes in addition to pyknotic nuclei(PK). The portal lumen of the pancreas is congested with blood and monocytes (40X)

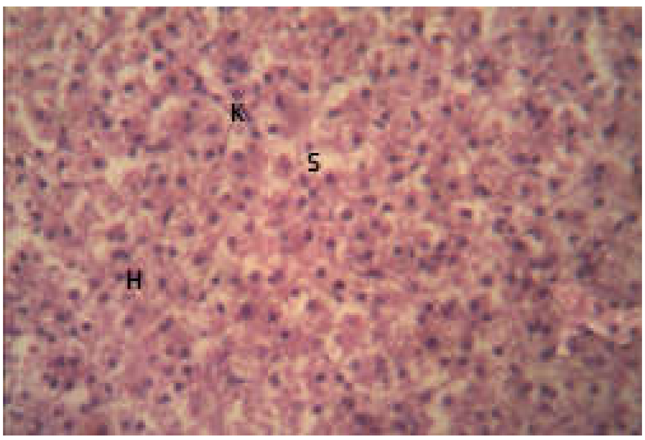

Fig:9.Section of liver of *Oreochromis mossambicus* fed with nanocurcumin formulated diet. Nearly, a normal architecture of the liver is detected. The hepatic portion is harboring polygonal hepatocytes. Sinusoids(S) are narrow and a spindle-shaped Kupffer cell(k) are seen. (40X)

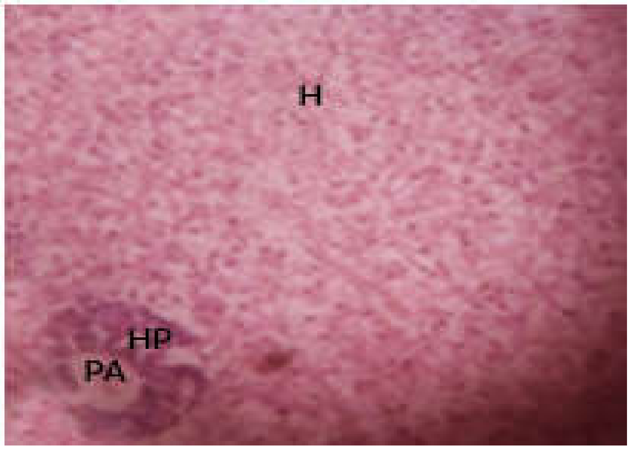

Fig. 10. Section of liver of *Oreochromis mossambicus* fed with nanotulsi incorporated diet.The hepatic part (H) exhibits a rather control like appearance (40X)

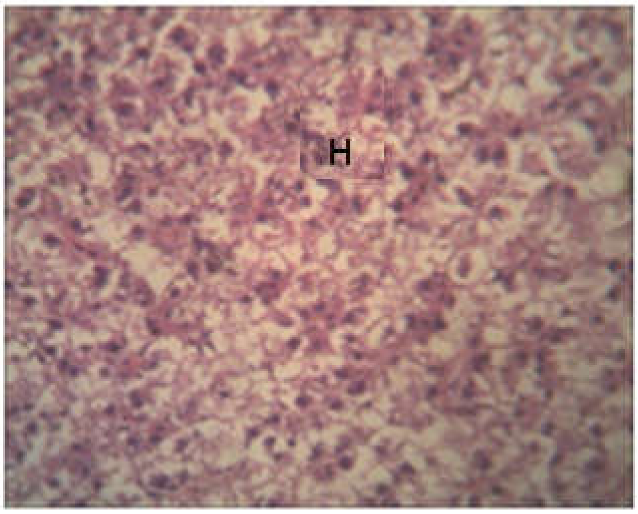

Fig. 11. Section of liver of *Oreochromis mossambicus* fed with aqueous extract of *Curcuma longa* showing the hepatic portion(H) crowded with polygonal hepatocytes possessing spherical nuclei and arranged radially around the central vein (CV).Sinusoids(S) are narrow and some spindle shaped kuppfer cells(K) are seen at one side of the endothelial linning of the sinusoid.(40X)

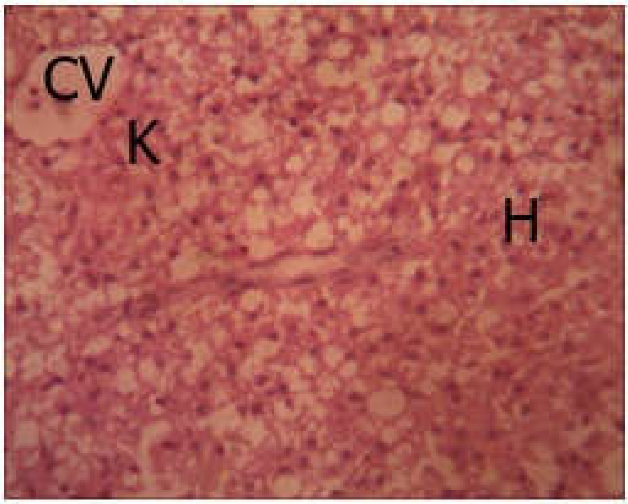

Fig. 12. Section of liver of *Oreochromis mossambicus* fed with aqueous extract of *Ocimum sanctum.*The hepatic part (H) is occupied by the usual polygonal hepatocytes with spherical nuclei. Kupffer cells (K) could be demonstrated at sides of the sinusoidal lumen (40X)

No histopathological alterations were observed in groups reared on diet containing aquous extracts of *Curcuma longa, Ocimum sanctum* and biogenic gold nanoparticles from the plant extracts. Good man *et al.,* 2004 Fig 9,10,11.12. Found that cationic gold nanospheres are toxic at certain doses. This observation was explained by the ability of cationic nanoparticle to interact with negatively charged cellular membrane and the resultant membrane disruption. Pan *et al* 2007; found that gold nanospheres triggered necrosis, mitochondrial damage and induced an oxidative stess on all examined cell line. The conflicting results could arise from the variability of the used toxic assays, cell lines and nanoparticle chemical physical properties. The histopathology slides of the present work suggested the presence of the gold Gold nanoparticles in the *Oreochromis mossambicus* tissues. No specific damage could be observed in the fish groups treated with biogenic nanoparticle incorporated diet. Hence in this study, cytotoxicity of biogenic gold is ruled out and it is confirmed that extracts of *Curcuma longa* and *Ocimum sanctum* along with gold nanoparticles at produce better cytostructural profile. These results clearly demonstrate that the phytochemicals within these herbs provide nontoxic coating on AuNps. The lack of any noticeable toxicity of biogenic gold nanoparticles provide new opportunities for the safe application in drug delivery. The results are in agreement with the work of Amit Singh *et al.* tittled" Cytotoxicity and Cellular Internalization Studies of Biogenic Gold Nanotriangles in Animal Cell Lines". The result becomes even more important when it is compared with the result of the same experiment using the plant extracts(*Curcuma longa* and *Ocimum sanctum*) alone. Thus, it shows that biogenic gold nanoparticles can be looked upon as an environmentally benign replacement to the toxic chemical methods for synthesis of nanostructures and as promising candidates for biomedical applications. Synthesized NPs can circulate in the body for extended periods of time without being rejected by the body's immune system. This can be exploited for benificial results by green synthesis of gold nanoparticle that avoids some reactive functional groups produced in chemically synthesized nanoparticles which can be toxic to biological system. The biogenic nanostructures should be better at biocompatibility and, thus, will have immense application for biological and clinical prospects

Conclusion

Despite increasing application of gold nanoparticles in industry and consumer products, there is still little known about their potential toxicity, particularly to organisms in aquatic environments. To investigate effects of gold nanoparticless in fish, tilapia *Oreochromis mossambicus* were supplemented with diet containing gold nanoparticles. Green synthesized gold nanoparticles showed little hepatic damage and showed better profile than the groups treated with plant extracts alone. The biogenic nanostructures are better at biocompatibility and, thus, will have immense application for biological and clinical prospects

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