Journal of Aquatic Biology and Fisheries Vol. 2/2014/ pp. 651 to 657

IMPACT OF BIOGENIC GOLD NANOPARTICLE ORAL ADMINISTRATION ON *OREOCHROMIS MOSSAMBICUS*

Shine, F*[1]., Akhilathomas, A[1]., Jayasree, S[1]., Sameeda[1], Joice S. George[1], Remya, C[1]., Shibu Joseph S.T[2].

[1]*Fisheries Biotechnology and Nano science unit ,P.G and research centre, Department of Zoology, Fatima Mata National College, Kollam 691001*

[2]*P.G, Department of Chemistry, Fatima Mata National College, Kollam.*

Email: shinecastelessf@gmail.com

Received on: 10 October 2013, accepted on: 12 December 2013

Abstract: Nanotechnology is a cutting edge area which amalgamates science and technology expertise to manipulate, measure, formulate, synthesise and monitor materials and devices at nanoscale (1-100 nm) dimensions. These structural novelties are coupled with a cocktail of functional symphonies which rightfully credits nanotechnology as a key driver to have influence on future industrial competitiveness of developed countries. The downsizing of matter to the nanometer level promises a plethora of opportunities ranging from information technology, environment and energy industry to health care, agriculture and fishery sector to name a few. Thus nanotechnology is projected as the next generation industrial revolution that is poised to revolutionize the present understanding of life and human society from all permissible angles. Gold (Au) exhibits unique and fascinating opportunities at the nanoscale when compared to its bulk form. Synthetic reducing agents are not favoured for synthesizing AuNPs for biomedical applications as traces of such chemicals left unreacted in the process can be harmful. Thus, alternative methodologies to replace synthetic chemical reducing agents for AuNPs preparation have recently been explored. Many plants and microbes have been found to be excellent sources of natural reducing agents. Biogenic gold nanoparticle have been used in the present study to understand its efficacy on oral supplementation in *Oreochromis mossambicus.* These gold nanoparticle were prepared using 0.3 M $HAucl_4 3H_2o$ (Sigma-Aldrich) and aqueous extract of *Emblica officianalis*. Synthesis of biogenic 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+- 0.35cm and 5+-0.62gm were collected from ADAK, Varkala, quarantined and stocked at 20 fish/1000L tanks and maintained at laboratory conditions. Experimental diets were prepared by incorporating 100 µl and 500µl of biogenic gold nanoparticle per 100gms of basal feed. Non-treated control diets and *Emblica officianalis* extract incorporated diets too were prepared. The experimental schedule was for six weeks and the fishes were fed at 4% of body weight twice daily. The biological effect was assessed in terms of microbial density alterations and biochemical turnover at selected sites. The microbiological and biochemical data revealed a negative correlation with respect to increase in biogenic treatments.The observations have been statistically analysed and discussed in the light of available literatu.

Key words: Gold nanoparticle, *Emblica officianalis,* Nano feed, Aqua culture, Nanotechnology

INTRODUCTION

Nanotechnology is revolutionizing human life in a major way over the past decade.Colloidal gold has been of prime interest to researchers and several works that have led to its potential application in the ûeld ofbiodiagnostics,(Rosi *et al*., 2005) therapeutics,(Pissuwan, 2006) drugdelivery, (Emerich *et al*., 2006) bioimaging, (Sharma *et al*., 2006) immunostaining, and biosensing.(Oloffson *et al*., 2003) Thus, the future use of gold nanostructures for various biological and clinical applications has been envisioned(Alivisators, 2001). Owing to the fact that metal nanoparticles have found a strong niche in the world of nanotechnology, there has been a constant con science effort to develop recipes for synthesis of metal nanostructures of varying sizes, shapes, and properties. However, recent biological syntheses of metal nanostructures have gained tremendous popularity due to the environmentally friendly green chemistry approach.Although a lot of work has been done to ascertain the cytotoxicity levels and biocom patibility issues related with gold nanoparticles, Shukla and coworkers gave the ûrst detailed account of the immunological response of cells on exposure to the gold nanoparticles and elucidated the mode of internalization.(Shukla *et al.*, 2005) They reported gold nanoparticles synthesized by sodium borohydride reduction did not show any visible cytotoxicity up to 100 µM concentration in macrophages. Furthermore, it was shown that gold nanoparticles did not elicit any stress induced production of pro-inflammatory cytokines TNFα or IL-1β in the macrophages. At higher concentrations, the gold nanoparticle inhibited the secretion of reactive oxygen species (ROS) and reactive nitrite species (RNS) and, thus, indicated that they have much to offer in the gold nanoparticle based biomedical applications Nanotechnology involves the application of materials at the nanoscale to new prod- ucts or processes. There are opportunities for the ûsheries and aquaculture industries to use existing nanotechnologies, and also to develop new applications speciûc to the industry. The potential beneûts of nanotechnology for ûsheries and aquaculture need to be balanced against concerns for the environment and the occupational health of workers.

Fishes will eat food containing nanomaterials and nanotechnology could be used to improve the delivery of micronutrients or unstable ingredients in aquafeeds. For example, the use of nanoencapsulation technology for fat-soluble vitamins, minerals and fatty acids may be advantageous. Some nanomaterials can change the physical properties (e.g. buoyancy, hardness) of the feed. For fish health in aquaculture, nanotechnological applications include antibacterial surfaces in the aquaculture system, nanodelivery of veterinary products in fish food using porous nanostructures, and nanosensors for detecting pathogens in the water.There are numerous potential applications of nano materials in aquafeeds. The addition of antimicrobial or antifungal agents (above) equally applies to preserving sacks of fish food

In recent years, gold nanoparticles (AuNPs) have be come prominent for its diverse applications . The initial step in the synthesis of AuNPs involves reduce tion of gold ions (Au1+ or Au3+) to neutral atoms (Auo) with a strong reducing agent. Synthetic reducing agents are not favoured for synthesizing AuNPs for biomedical applications as traces of such chemicals left unreacted in the process can be harmful. Thus, al ternative methodologies to replace synthetic chemical reducing agents for AuNPs preparation have recently been explored. Many plants and microbes have been found to be excellent sources of natural reducing agents

In the present study, the plant selected for biogenesis of gold solution is *Emblica officianalis*. The species is well known for its many remedialproperties. Synthesized nano particle is confirmed by the change of color of Auric chloride which is yellow in color, and growth of nano paricle was monitored by surface plasmon behavior using UV-Vis Spectroscopy and concerned pH was determined. Furthermore, this green synthesis approach is rapid and better alternative to chemical synthesis

Gold in nano scale display novel properties and have diverse activities make it appropriate for therapeutic uses and broad applications in nanobiotechnology. Phytochemical constituents in the plants and spices extract like essential oils (terpenes, eugenols, etc), polyphenols and carbohydrates these compounds contain active functional groups, such as hydroxyl, aldehyde and carboxyl units which may play important role for reduction of HAuCl4 to AuNPs. Gold nanoparticles producedby using phytochemicals or other extract components remain stable for certain time.Further plants and spices mediated stabilized or capped AuNPs may cross the barrier of cytotoxicity which is a prior requirement for biomedical application of AuNPs).

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; Boon *et al.*, 2002). Due to exposure to pollutants, major structural damages occur in their target organs, histological structure may change and physiological stress may occur. This stress causes some changes in the metabolic functions

The objective of the study is to determine the acute side effects of biogenic gold nanoparticle in the aquatic environment on Mossambic tilapia (*O. mossambicus*) with emphasis on micro biological and biochemical parameters.

MATERIALS AND METHODS

Synthesis of Biogenic gold Nanoparticles

Emblica officianalis fruit were washed with double distilled distled water and shade dried before being grinded to fine powder and sieved to remove coarse particles. 1 gm. E.O was mixed with 100 ml of doubled distilled distled water and the mixture was left in a shaking incubator operating at 200 rpm, 25°C for 24 h. The extract was then filtered and the filtrate used for AuNPs synthesis. 1ml of the aquous extract of *Emblica officianalis* were mixed with various concentrations (100,500 µl) of $HAuCl_4$ (1 ml) and the reaction volume was made upto 2 ml with double distilled water. The mixture solution was left on constant magnetic stirring at room temperature (29°C) and observed for change in colour.

Research grade $HAuCl_4.3H_2O$ (Sigma) at 0.28M was used for the reduction process. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (Dynamica HALO DB -20) at the range of 200-800 nm and observed the absorption peaks *at 530-550 nm* regions due to excitation of surface plasmon vibrations in the Au NPs solution which are identical to the characteristics uv visible spectrum of metallic gold and it was recorded. (Fig. 1)

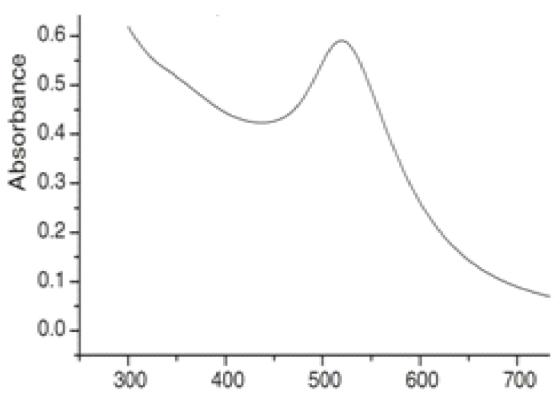

Fig. 1. UV-Vis absorption spectrum of pure Gold nanoparticles. The peak at 520 nm is due to the plasmon resonance of pure Gold colloid.

Experimental Design

Juveniles of *Oreochromis mossambicus* in the range 7ï0.35cm and 5ï0.62gm were collected from ADAK, Varkala, quarantined and stocked at 20 fish/1000L tanks and maintained at laboratory conditions.Basal feed was prepared as out lined by Hardy *et al.* (1978). Experimental diets were prepared by incorporating 100 µl and 500µl of biogenic gold nanoparticle per 100gms of basal feed. Non-treated control diets and *Emblica officianalis* extract incorporated diets too were prepared .The experimental schedule was for six weeks and the fishes were fed at 4% of body weight twice daily. The biological effect was assessed in terms of microbial density alterations and biochemical turnover at selected sites.

The total bacterial load in the gill, gut and skin was assayed as per the techniques outlined by Kannan *et al* (2002). In biochemical analysis total protein in the tissues (gill,gut,skin,muscle and liver) were assayed by Folin cio-calteu reagent using Bovine serum albumin as standard (Lowery *et al.*, 1951). The glycogen content in the tissue was estimated by Anthrone method following Sifter *et al.* (1950) the total Lipid content was estimated by Chloroform Methanol method.

RESULTS AND DISCUSSION

Antimicrobial Analysis

The total bacterial load in the gill, gut and skin was assayed as per the techniques outlined by Kannan *et al.* (2002).

As per the experimental design mentioned in the materials and methods, the result obtained are presented in Table 1. The data depicts the total bacterial load at gill, gut and skin of both control and treated *Oreochromis mossambicus*. Microbial density of selected sites revealed significant fall in the treated group.

The Colony Forming Units for biogenic gold nanoparticulates (500µl) supplemented group was 25×10^8 CFU/g,80×10^8 CFU/g and 104×10^8CFU/g for gill,gut and skin respectively.The same for the control group was223×10^8 ,316×10^8 and 326×10^8 for gill, gut and skin respectively. The values of those group fed with aquous extract of *Emblica officianalis* added

Table 1. The total bacterial load at gill, gut and skin of both control and treated *Oreochromis mossambicus*.

CONTROL	GILL	GUT	SKIN
Biotransformed Gold Nanoparticle(100 µl)	223×10^{8}	316×10^{8}	326×10^{8}
Biotransformed Gold Nanoparticle(500µl)	25×10^{8}	80×10^{8}	104×10^{8}
Emblica officianalis	128×10^{8}	218×10^{8}	344×10^{8}

feed was 128×10^{-8}, 218×10^{-8} and 344×10^{-8} for gill, gut and skin respectively. The CFU values for the group fed with biogenic gold containing 100 µl of gold involves11 5×10^{8} for gill, 197×10^{8} for gut and 220×10^{8} for skin.

Severe antimicrobial impact was observed at all the sites examined.The present findings indicates considerable drop in the microbial loads in the groups exposed to gold nanoparticulated feed. Dose dependent decrease in microbial load was found with the concentrations 500 µl and 100µl of biogenic gold nanoparticle and can concluded that biotransformed gold nanoparticle has profound antimicrobial activity. The results also reveals explicitly the antimicrobial property of *Emblica officianalis* which gets compounded on biotransformation when compared to the fish groups fed with basal feeds.

A large number of herbs are known to contain strong antibacterial activity that can be used to control different fish disease.Dose dependent decrease in the microbial load was elucidated Jamine et;al in Macrobrachuim rosenbergii with Ocimum sanctum. Ferdory et.al 2011 claimed that juice of *Citrus aurantofolia*, extract of allium sativum and leaf extract of *Lawsonia inermis* and *Emblica officianalis* had inhibitory effect for edwardsilla species isolates collected from diseased infected fish. Gosh *et al*. (2011) conducted screening effect of antibacerial activity of plant evtracts and reports that both aqueoue and methanolic extract of *Terminalia chebula*, *Polyalthia longifolia*, and *Emblica officianalis* were reffective for bacterial strain *P. fluorescens* isolated from epizootic ulcerative syndrome diffected fish.These reports support our finding of increase of antimicobial effect of *Emblica officianalis* treated fish when compared to the control. Antimicrobial activity of algae mediated synthesis of gold nano particle from *Turhinaria conoides,* shows streptococcus species having the maximum inhibition and medium range of inhibition were examined against *B. Subtilis*. In the year of 2012 Nagari et al investigated effect of gold NP against *P.aerugenosa, Staphylococcus aureus,* E.coli. The study also shows increased zone of inhibition at high concentration of gold NP. The present research work was undertaken to access the antimicrobial effect of biotransformed gold nanoparticle with *E.officianalis* on microbial parameter of *O. mossambicus*. The result proves the antimicrobial activity of *E. Officianalis* and also reveals the biotransformed gold nano solution enhances the antimicrobial activity . Further assesment for undertaking the mechanisms involved in enhanced immuno competence of the physical bacteria would be a promising research area .

Severe antimicrobial impact was observed at all the sites examined by Jayasree *et al*. (2012a). They have discussed the in vivo impact of Triphala supplementation on *Oreochromis mossambicus*. Herbal medications have the potential to alter the microbial diversity for the betterment of culture fishes according to them. Nanoparticle effect on biological models is yet to gather momentum.(De Azeredo, 2009). Aquaculture applications of NP is at present revolving around water treatment and antimicrobial efficacy (Freitas, 2005). In vivo antimicrobial effect of gold nanoparticles of 15nm size and 0.005 µg/L was assessed by Jayasree *et al* (2012b). They have reported severe antimicrobial effect at the tissue analysed . The present findings indicates considerable drop in the microbial loads in the groups exposed to gold nanoparticles and can concluded that gold NP has prefound antimicrobial activity. The results also substantiate well the antimicrobial property of *Emblica officianalis* coupled with gold nanoparticle when compared to the fish groups fed with basal feeds.

Biochemical Analisis

Total protein in the tissues (gill,gut,skin,muscle and liver) were assayed by Folin-cio-calteu reagent using Bovine serum albumin as standard.(Lowery *et. al*.,1951) The glycogen content in the tissue was estimated by Anthrone method following Sifter *et al*. (1950) the total Lipid content was estimated by Chloroform Methanol method.

As per experimental design mentioned in the materials and methods, the result obtained are depicted in the Tables 3 to 6.

The biochemical turn over as reflected by the protein, glycogen and lipid content of a vital tissue gives an understanding of assimilation, utilization of these molecules. The mean protein content (n=6) of control fish are muscle-15.34 mg%, gill-4.56 mg%, gut - 4.17 mg%, liver - 4.52 mg% and skin - 8.89 mg% respectively. Total glycogen content of various tissues in mg % are as follows, liver - 7.45, muscle - 1.74, skin - 0.772, gill - 0.721 and gut - 0.531. muscle - 0.523 mg% and gut - 0.423 mg% respectively. The lipid content (mg%) being gill-0.569, gut-0.423, liver -4.07, Muscle -0.523, skin-0.878, respectively.

Table 2. Biochemical content at certain tissues of *Oreochromis mossambicus* rared on basal diet

CONTROL			
Sample (n=6)	Protein (mg%)	Glycogen (mg%)	Lipid (mg%)
Gill	4.56±0.13*	0.721±0.09	0.569±0.02
Gut	4.17±0.15	0.581±0.03	0.423±0.06
Liver	4.52±0.10	7.45±0.21	4.07±0.31
Muscle	15.34±2.07	1.74±0.01	0.523±0.02
Skin	8.89±1.06	0.772±0.08	0.878±0.09

Table 3. Biochemical content at certain tissues of *O. mossambicus* fed aquous extract of *Emblica officianalis*.

Emblica officianalis			
Sample (n=6)	Protein (mg%)	Glycogen (mg%)	Lipid (mg%)
Gill	12.60+.0.4	2.95+.0.55	0.390+.0.07
Gut	5.41+.0.71	3.68+.0.16	0.298+.0.71
Liver	6.48+.0.31	2.93+.0.05	0.265+.0.02
Muscle	12.34+.0.98	1.88+.0.11	0.236+.0.03
Skin	13.61+.0.31	2.97+.0.06	0.244+.0.4

Table 4. Biochemical content at certain tissues of *O. mossambicus* fedbiotransformed Gold nano particle (100µl) feed.

GOLD NANO TREATED(100 µl)			
Sample (n=6)	Protein (mg%)	Glycogen (mg%)	Lipid (mg%)
Gill	12.98±1.23	8.50±0.32	0.254±0.06
Gut	8.36±0.59	7.82±1.03	0.238±0.04
Liver	12.87±2.01	10.54±1.16	0.223±0.09
Muscle	13.04±1.98	6.46±0.76	0.219±0.01
Skin	14.63±2.09	7.14±1.87	0.316±0.07

Table 5. Biochemical content at certain tissues of *O. mossambicus* fed-biotransformed Gold nano particle (500µl) feed.

GOLD NANO TREATED (500µl)			
Sample (n=6)	Protein (mg%)	Glycogen (mg%)	Lipid (mg%)
Gill	4.625±1.65	1.44±0.21	0.313±0.49
Gut	4.310±0.38	2.49±0.33	0.218±0.43
Liver	5.391±0.21	1.45±0.27	0.284±0.32
Muscle	6.626±1.04	2.22±0.33	0.282±0.11
Skin	7.562±1.55	1.64±0.39	0.297±0.09

The total protein content of *Emblica officianalis* treated fish are, muscle – 12.34mg%, gill – 22.60mg%, gut – 15.41mg%, liver – 16.48mg% and skin -15.61mg% respectively. Total glycogen content of various tissues in mg% are as follows, liver -2.93, gill -2.95, gut -3.68, muscle – 1.88and skin -2.97. The lipid content in the liver – 0.265mg%, muscle - 0.236 mg%, gut - 0.298 mg%, gill - 0.390 mg% and skin - 0.244 mg% respectively.

The total protein content of gold nano (100 micro litre) treated fish are gill – 12.98mg%, gut – 8.36mg%, liver – 12.87mg%, muscle – 13.04mg% and skin – 14.63mg%respectively. The glycogen content of various tissues in mg% are as follows, gill -8.50, gut-7.82, liver -10.54, muscle – 6.46and skin -7.14. The lipid content in the gill 0.254mg%, gut - 0.238 mg%, liver - 0.223 mg%, muscle - 0.219 mg% and skin - 0.316 mg% respectively.

The total protein content of gold nano (500 micro litre) treated fish are gill – 4.625 mg%, gut – 4.310 mg%, liver – 5.319 mg%, muscle –

6.626 mg% and skin – 7.562 mg% respectively. The glycogen contend of various tissues in mg% are as follows, gill - 1.44, gut - 2.49, liver - 1.45, muscle - 2.22 and skin - 1.64. The lipid content in the gill – 0.313 mg%, gut - 0.218 mg%, liver - 0.284 mg%, muscle - 0.282 mg% and skin - 0.297 mg% respectively

A dose dependent decrease in the biochemical parameter in various tissues indicate the toxicity of biotransformed gold nanopartical in *E. officianalis* extract. Medicinal plants and their products can be viewed as an important commodity item for sustainable aquaculture development of the country (Rao and Chakrabati, 2005). In the work done by Jibi (2006) "Studies on the immunomodulatory effect of *E. officianalis* of extract on *O. mossambicus*" stated conclusively that *E. officianalis* has a growth promoting and immunomodulatory effect on *O. mossambicus*. Thus from the present study we can assume that the toxic effect of *E. officianalis* is due to the biotransformed gold NP present in it. Ahammed, Riswan and Chand in their work titled "Cytoprotective effect of *E. officianalis* against Endosulfan induced toxicity in liver of *Clarias batrachus*"; found perfect correlation between biochemical and histopathological findings.

Much more to be studied to understand the mechanisms behind the toxicity of biotransformed gold NP in *O. mossambicus*. Since toxicity decreases with decreasing dose of gold NS concentration (in present study 500 µl and 100 µl) further studies should be proceeded with lower concentration of gold nano solution to employ the green synthesized gold nano solution as possible therapeutant.

CONCLUSIONS

There are numerous potential applications of nanomaterials in aquafeeds. The addition of antimicrobial or antifungal agents equally applies to preserving sacks of fish food. However, nanotechnology may offer some significant advantages in the delivery of micronutrients or other less stable ingredients to the Fish. Nanomaterals may be used to enclose or coat (nanoencapsulation technology) nutrients that would normally degrade, such as fatty acids, or have limited assimilation efûciency across the gut of ûshes, because they are poorly soluble (e.g. fat-soluble vitamins) In addition to improving the bioavailability and stability of the food ingredients, nanomaterials may be used to alter the physical properties of ûsh food. Small additions of nanomaterials can dramatically change the physical properties of food pellets.

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. The applications of nanoparticles in disease diagnosis and therapy , it is important that the systems are biocompatible and capable of being functionalized for recognition of specific target sites in the body after systemic administration.In the present study since toxicity decreases with decreasing dose of biogenic gold concentration(500µl and 100µl) further studies should be undertaken with lower concentration of biotransformed gold nano solution to employ the green synthesized gold nanosolution as possible therapeutant. The results from the antimicrobial study proves the antimicrobial activity of gold nanoparticles. The total microbial load in the gill.,gut and skin of nanoparticle treated fish are comparatively very low than control.The results reveals well the antimicrobial effect of *Emblica officianalis* also.

REFERENCES

Ahammed, Riswan and Chand. 2009. *Journal of Ecophysiology and Occupational health,* 9: p153

Alivisatos, A.P. 2001. Less is more in medicine - sophisti cated forms of nanotechnology will ûnd some of their ûrst real-world applications in biomedical research, disease diagnosis and, possibly, therapy. *Sci. Am*., 285: 66–73

De Azeredo, H.M.C. 2009. Nanocomposites for food packaging applications. *Food Res.Intl*., 42: 1240-1253

Emerich, D.F., Thanos, C.G. 2006. The pinpoint and molecular diagnosis. promise of nanoparticle-based drug delivery, *Biomol, Eng*., 23: 171–184.

Freitas, R.A. 2005. what is nanomedicine,1,2-9.

Handy, R., von der Kammer, F., Lead, J., Hassell, M., Owen, R. and Crane, M. 2008a. *The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicology,* 17: 287-314.

Hardy, R. 1978. *Fish feed formulation in fish feed technology lecture -9 oct-dec;* pp. 233-238.

Hardy. R. 1978. Fish feed formulation in fish feed technology lecture-9.oct-.dec; pp. 233-238

Jayasree, S.A., Akhila Thomas, Sameeda, S., Shibu Joseph, S.T., Jibi, S. and Remya, V. 2012b. Silver nanoparticle exposure on biochemical turn over and microbial alterations I oreochromis mossambicus. Paper presented at National Conference on recent trends in molecular biology.

Jayasree, S.A., Akhila Thomas, Sameeda, S. Shine, F. 2012b. Fluctuations in the microbial diversity at certain tissues of Oreochromis mossambicus supplemented with triphala. National Seminar on Biotechnology and Environment.

Kannan, N. 2003. Hand book of laboratory culture media, reagents, stain and buffers; *Panima publishing Co, New Delhi*.

Lowry, O.H., Roseborough, N.T, Farr, A.l, and Rand ell, R.J. 1961. Protein measurement with Folin phenol reagent. J.Biol.Chem., 193: 263-265.

Olofsson, L., Rindzevicius, T., Pfeiffer, I. and Kall, M. 2003. Hook, F. Surface-based gold-nanoparticle sensor for specific and quantitative DNA hybridization detection. *Langmuir*, 19: 10414–10419.

Pissuwan, D., Valenzuela, S. M. and Cortie, M.B. 2006. Ther apeutic possibilities of plasmonically heated gold nanoparticles. *Trends Biotechnol*., 24: 62–67.

Rao, Y.V. and Chakrabarti. R. 2005. Stimulation of Immunity in Indian major Carp *Catla catla* with herbal feed ingradients. *Fish and shell fish immunology.* 18: 327-334

Rosi, N.L. and Mirkin, C.A. 2005. Nanostructures in biodiagnostics. *Chem. Rev.*, 105: 1547–1562

Sifter, S., Dayton, S. and Naik, B. 1950. "Colorimetric determination of glycogen by Anthrone method in tissue". *Arch biochem*., 25: 191-200

Sharma, P., Brown, S., Walter, G., Santra, S. and Moudgil, B. 2006. Nanoparticles for bioimaging. Adv. Colloid Interface Sci., 123–126, 471–485.

Shukla, R., Bansal, V., Chaudhary, M., Basu, A., Bhonde, R. R. and Sastry, M. 2005. Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: A microscopic overview. *Langmuir*., 21: 10644–10654.