E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2020; 8(2): 277-284

www.fisheriesjournal.com
Received: 16-01-2020
Accepted: 18-02-2020

Dr. Shivaraj Yallappa
Assistant Professor, Department of Zoology, L.V.D College Raichur, Karnataka, India

Kaveri KR
Full-Time Guest Faculty, Department of Studies & Research in Zoology, Tumkur University, Tumakuru, Karnataka, India

Dr. Asiya Nuzhat FB
Associate Professor & Co-Ordinator, Department of Studies & Research in Zoology, Tumkur University, Tumakuru, Karnataka, India

Dr. Vijaykumar K
Vice-Chancellor, Gulbarga University, Kalaburagi, Karnataka, India

Corresponding Author:
Dr. Shivaraj Yallappa
Assistant Professor, Department of Zoology, L.V.D College Raichur, Karnataka, India

Toxic effect of green synthesized silver nanoparticles on freshwater fish tilapia, *Oreochromis mossambicus* (Peters)

Dr. Shivaraj Yallappa, Kaveri KR, Dr. Asiya Nuzhat FB and Dr. Vijaykumar K

Abstract
An eco-friendly synthesis of silver nanoparticles (AgNPs) from silver nitrate solution using aqueous Apple fruit extract was investigated. The reduction of silver ions in solution was monitored using UV–visible absorption spectroscopy and morphological analysis was studied by transmission electron microscopy. Short term definitive test by static renewal bioassay method was conducted to determine the acute toxicity (LC_{50}) of green synthesized silver nanoparticles on freshwater fish fingerlings were exposed to different concentrations and the fixed doses 50mg/L for 96hrs, 100mg/L for 96hrs, 150mg/L for 96hrs, 200mg/L for 96hrs and 250mg/L for 96hrs were selected for acute study on fish Tilapia, *Oreochromis mossambicus* procured from Fisheries Research and Information Centre (Inland) Hebbal, Bangalore, Karnataka, India. The size of the fish was (30 ± 35 g; 13 ± 14 cm) and duration 4 days respectively. Further investigation, the green synthesized silver nanoparticles on the changes in behaviour and bodyweight of a freshwater fish, Tilapia, *Oreochromis mossambicus.* The results of the study evidenced that green synthesized silver nanoparticles is non-toxic but it has led to the altered fish physiology for long term of exposure. However the exact mechanism through which this is achieved needs to be studied further.

Keywords: Green synthesis, AgNPs, characterization, toxicity studies and *Oreochromis mossambicus* (Peters)

Introduction
Nano science and technology is one of the rapidly growing fields as it has potential applications in the fields of information and communication technologies, biotechnology and medicine, optoelectronics and solar-cell. Nano science and nanotechnology primarily deal with the synthesis, characterization and exploration of various kinds of nanostructured materials. Nanostructures constitute a bridge between molecules and infinite bulk systems. Individual nanostructures include clusters, quantum dots, Nano crystals, nanowires, and nanotubes while collections of nanostructures involve arrays, assemblies, and super lattices of the individual nanostructures (Rao C N *et al.*, 2001) [40]. The uniqueness of the structural characteristics, energetics, response, dynamics and chemistry of nanostructures constitutes the basis of Nano science. In general, the nanomaterial have very large surface area to volume ratio due to their small size which brings out new physical and chemical properties compared to their bulk counterparts. The mechanisms of the property change in the material include size dependent quantum confinement effects, change of dimensionality of the system and so on. The properties of materials with nonmetric dimensions are significantly different from those of atoms or bulk materials. The Nano science and technology have grown rapidly especially from the last two decade, due to the ease of use of advanced characterization techniques as well as large number of synthesis methods for nanomaterial.
Nanoparticles can be broadly classified into two groups: Organic nanoparticles and Inorganic nanoparticles. Organic nanoparticle are carbon nanoparticle (fullerenes) and inorganic nanoparticles are magnetic nanoparticle, noble nanoparticle (Au and Ag), semiconductor nanoparticle (TiO2 and ZnO). Especially inorganic nanoparticles have created attention towards itself due to its superior material properties with versatile functions. Due to nano size feature it is easily used for chemical imaging drug agents and drugs.

Its versatile function is used for the cellular delivery as they are widely available, rich functionality, good biocompatibility. This is also a good carrier of targeted drug delivery and controlled drug release (Xu *et al.*, 2006) [43]. It is a completely advantageous material for medical science (eg: mesoporous silica combined with molecular medicines shows an excellent image on drug releasing). Gold nanoparticle is good carrier in thermo therapy of biological target. Silver nanoparticle shows antimicrobial activity which heals the wounds and infectious disease. Synthesis of nanoparticle gets concern in nanotechnology due to the variable size, shapes, chemical composition and controlled inequality and their potential use in the medical science for the better treatment of human benefits.

Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanomaterials is an important aspect of current nanotechnology research. One of such promising processes is green synthesis. Silver nanoparticles can be synthesized by several physical, chemical and biological methods. However for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality. Silver nanoparticles (AgNPs) have been receiving broad interest for a large number of applications such as in optics (A. Sivanesan, H K *et al.*, 2011) [1], selective coatings for solar energy absorption (K. Liu *et al.*, 2013) [20], bio labeling (H. Zhu *et al.*, 2013) [15], catalysts (P. Zhang *et al.*, 2011) [38] and antibacterial agents (W. A Ismail *et al.*, 2013) [42] owing to their unique properties. In antibacterial application, for instance, apart from being effective, AgNPs still remain a popular choice due to their nontoxicity towards human (M. Rai, A. Yadav and A. Gade 2003) [29] in comparison to other metals or materials. However, scarcity makes them expensive and limits their application. To overcome the problem, numerous synthesis methods have been developed (K. K Caswell *et al*., 2003, Y.Yin *et al.*, 2002, P. K Khanna *et al.*, 2005, L. Maretti *et al.*, 2009, B. Wiley *et al.*, 2004 and M. D Malinsky *et al.*, 2001) [19, 44, 37, 24, 6, 28]. Most of the conventional methods for producing AgNPs require numerous chemicals, which not only is expensive but also could produce hazardous residue. Therefore, a green synthesis of AgNPs is desirable to provide an economic, eco-friendly, and cleaner synthesis route. A number of biomolecules in extracts have been shown to successfully act as reducing agents in the green synthesis of AgNPs. For example, black tea leaf extract has been used for the biosynthesis of AgNPs with sizes averaging 20nm (M. J Uddin 2012) [30]. The extract of *Mangifera indica* leaf also produces AgNPs with sizes of about 20nm (D. Philip 2011) [9]. Extracts from fruits such as the red fruits of the piquin pepper (*Capsicum annuum var. aviculare*) have also been shown to produce AgNPs in the range of 3–10 nm (R. Mendoza-Res´endez *et al.*, 2013) [39]. The aqueous extract of *Hovenia dulcis* fruit produces AgNPs with sizes of 45nm (N. Basavegowda *et al.*, 2014) [33].

Green synthesis of nanoparticles

The improvement of resourceful green chemistry methods are employing for natural reducing capping and stabilizing agents to organic silver nanoparticles through preferred morphology and size have considered as foremost focus of researches. The green synthesis methods using plant and microorganisms have been employing to synthesis of silver nanoparticles. Some of them are used microorganism such as fungi (Ahmad *et al.*, 2003) [2], bacteria (Klaueh *et al.*, 2000) [22] and actionmycetes. However the rate of the synthesis is slow and only limitations such as number of sizes, shapes and difficulty of completion on a large scale and they require for maintaining cell cultures are amenable compared to routes involving plant based materials. Plants parts such as leaf, stems roots (Ahmad *et al.*, 2010) [3], seeds (Bar *et al.*, 2009) [7], latex (Bar *et al.*, 2009) [7], gum and fruits (Li *et al.*, 2008) [25] are being used for metal nanoparticles synthesis. Plant based materials seem to be the best candidates and they are large-scale production nanoparticles and also eco-friendly alternatives to physical and chemical methods. The major advantage of using plants for the synthesis of silver nanoparticles is that they are easily available, safe to handle, non-toxic and have a broad change in the metabolic process of organisms and in support of toxicants reduction. The era necessary for 90% of the reduction of silver ions was on 2 or 4hrs. The main mechanism of overall observation clearly indicates that biological materials provide an environment friendly and produce invaluable materials because these methods eliminate from harsh or toxic chemicals.

Significance of green synthesis

The major approach for biological synthesis or green synthesis of silver nanoparticles is regarding biocompatibility and environmental toxicity (Kumar *et al.*, 2008) [23]. The further advantage of this approach is as follows:

1. No formation of toxic substances and also behave as environmental friendly.
2. In green synthesis process, there is no addition of any chemical agent for reducing and capping purpose.
3. The antioxidant or reducing properties of biomolecules of the organism reduces the metal to form nanoparticles.
4. Cost effective.
5. No use of high pressure, temperature, energy and toxic chemicals.
6. Can be easily scaled for large scale synthesis.

The Silver nanoparticles (AgNPs) are being released into aquatic system due to their extensive use in daily human activities. The accumulation of AgNPs in aquatic biotic components would be consumed by higher order trophic animals. This leads to the transfer of AgNPs to food chain which will imply the risk for human health (Jiang H.S *et al.*, 2017) [18]. Even though the AgNPs are widely used, there is little information available on the long term toxic effects on human health as well as ecology. The AgNPs in higher concentrations may be toxic and can induce health risks and cause disturbance in the ecosystem (Sukumaran Prabhu and Eldho K Poulose 2012) [41]. Being healthy food for human, there should be strong concern about the toxic effect of AgNPs on aquatic animals especially on fishes also. The toxicological risks connected with nanoparticles are partially known. The recent studies reported the toxic effect of nanoparticles on fish (King Heiden T.C *et al.*, 2007, Muhammad Saleem *et al.*, 2015) [21, 32]. In spite of numerous studies in fish but more studies are required to illustrate the risks associated with toxicity of nanoparticles on fish and finally this present investigation shows the toxic effects of green synthesized silver nanoparticles on freshwater fish Tilapia, *Oreochromis mossambicus* (Peters).

Materials and Methods

Synthesis of silver nanoparticles

The ultra-pure silver nitrate and sodium borohydride are purchased from the S D fine-chem limited. To prepare 1mM AgNO3 solution, 0.0169g of silver nitrate is weighed and transferred into 250ml conical flask and dissolved by adding 100ml distilled water and stirred well. The 0.075gm of NaBH4 is weighed and dissolved in 100ml distilled water. The concentration of the prepared sodium borohydride solution is 2mM. The sodium borohydride solution is prepared freshly during the time of synthesis of silver nanoparticles. Silver nanoparticles are prepared by the Turkevich method. 30mL of 2mM aqueous solution of sodium borohydride (NaBH4) is taken in a clean 250ml conical flask. The conical flask containing NaBH4 solution is kept on the ice bath to maintain the temperature. The NaBH4 solution is vigorously stirred using magnetic stirrer for minutes and then slowly added to the 2ml of 1mM AgNO3 solution dropwise. When 2ml of AgNO3 solution is added to the ice cooled NaBH4 solution, the solution is turned into golden yellow colloidal solution. The colloidal solution of silver nanoparticles is collected in well-sealed glass container for characterization and antimicrobial study.

Characterization of silver nanoparticles

The UV-visible spectroscopy is found to be one of the fastest characterization techniques of nanoparticles (Jayshree Annamalai & Thangaraju Nallamuthu 2016) [17], (Madanagopal Nalini *et al.*, 2017, Dwivedi D. K *et al.*, 2010) [31, 12]. Silver nanoparticles show the surface plasmon resonance absorption due to the phenomenon of mutual vibration of electrons in the silver nanoparticles when excited by the electromagnetic radiation (Anandalakshmi K., Venugobal J & Ramasamy V 2016, Noginov M.A *et al.*, 2006, Link, S and El-Sayed M.A. 2003) [4, 35, 27]. The UV-visible spectrum of AgNPs is recorded using the Eppendorf Bio-Spectrometer Kinetic and the synthesis of AgNPs is further confirmed by Transmission Electron Microscope (TEM), the JEM-1400 is available at Indian Institute of Technology, Delhi. The JEM-1400 Transmission Electron Microscope has a maximum accelerating voltage of 120 KV.

Collection of fishes, and their maintenance

The collection of fish, from fisheries research and information center (inland) Hebbal, Bangalore, Karnataka, India provided healthy and active 6-8 months old Tilapia, *Oreochromis mossambica* (30±35 g; 10±14). Large aerated crates were used to transport the fishes to the laboratory. Before investigation fishes were maintained for 7 days in large cement tank (22×12×5 Feet). Further, 50 fishes were acclimatized to laboratory condition for 7 days at 26±2 ºC in 30 L capacity plastic tubs containing tap water is used for experiment. Characteristics of water were determined by following the methods mentioned in APHA (2005) [5] which are presented in Table 1.

Fig 1: Tilapia, *Oreochromis mssambicus* (Peters) 1852

Table 1: Physico-Chemical Characteristics of Water Quality used in this Present Study.

Variable	Datum
Temperature	26±2 ºC
pH	5 at 26 ºC
Dissolved oxygen	6±7 mg/L
Conductivity	450 µS/cm

Water was renewed every day with 12 hours dark cycle and 12 hours light cycle was maintained and fish were fed (ad-libitum) daily with commercial dry feed pellets (Nova, Aquatic P. Feed) during acclimatization periods and test periods control fishes were feed but in toxicity testing, feeding was stopped one day prior exposure to the test medium. During acclimation batches with less than 5 % of mortalities were only considered for further experimentation.

Acute Toxicity Test (LC_{50})

The acute toxicity (96 hr LC_{50}) of Silver Nanoparticles for the freshwater fish, Tilapia, *Oreochromis mossambicus* was determined in the laboratory using the semi-static method in OECD. (1992) [36]. The carp (10 fingerlings in 20 L of test medium in each replicate) was exposed to varying concentrations of industrial effluent with two replicates for each concentration along with the control sets. Test medium was renewed for every 24 hr with their respective test concentrations of the toxicant without aeration. Mortality was recorded every 24 hr and the dead fish were removed when observed, every time noting the number of fish death at each concentration up to 96 hr for estimation of acute toxicity (LC_{50}). Time of exposure was the repeated measure factor while treatment (concentration and control) was the second factor. The LC_{50} was calculated using probit analysis (Finney O.J 1953) [13] which has been recommended by OECD guideline as an appropriate statistical method for toxicity data analysis (Lilius H. A 1994) [26]. After linearization of the concentration response curve by logarithmic transformation of concentrations (log+2), 96 hr LC_{50} with 95% confidence limits and slope function was calculated to provide a consistent presentation of the toxicity data.

Experimental design and test concentrations

In this experiment lethal concentrations of green synthesized silver nanoparticles 50mg/L for 96hrs, 100mg/L for 96hrs, 150mg/L for 96hrs, 200mg/L for 96hrs, 250mg/L for 96hrs were selected for acute study. Each replicates possessed 10 fishes in 20 L of the test medium and two replicates group-1 (control) and group-2 (treated) were maintained for each concentration and control. Tilapia, *Oreochromis mossambicus* were exposed the test concentration for 4 days of time period but 96hrs for each group were exposed the concentration levels of each group were 50, 100, 150, 200 & 250mg/L increased up to the motility arises in this level of exposure and were allowed to recover in toxicant free medium for seven days. In this experiment periods test medium was renewed daily followed by addition of respective test concentrations of Green synthesized silver nanoparticles on up to day 4 without aeration, respectively.

Study of Behaviour and Morphological Changes

In this investigation monitoring of test species is performed at regular time intervals (8 hrs) to observe and record the spectrum of behavioral responses and morphological deformities in both the control (toxicant free medium) and

Green synthesized silvernano particles treated fish. Photographs of test species were acquired using camera phone (Moto M) to delineate different phase. Fish were then photographed to capture the course and different phases of behavioral as well as morphological deformities.

Results

Graph of UV absorbance spectrum of AgNPs.

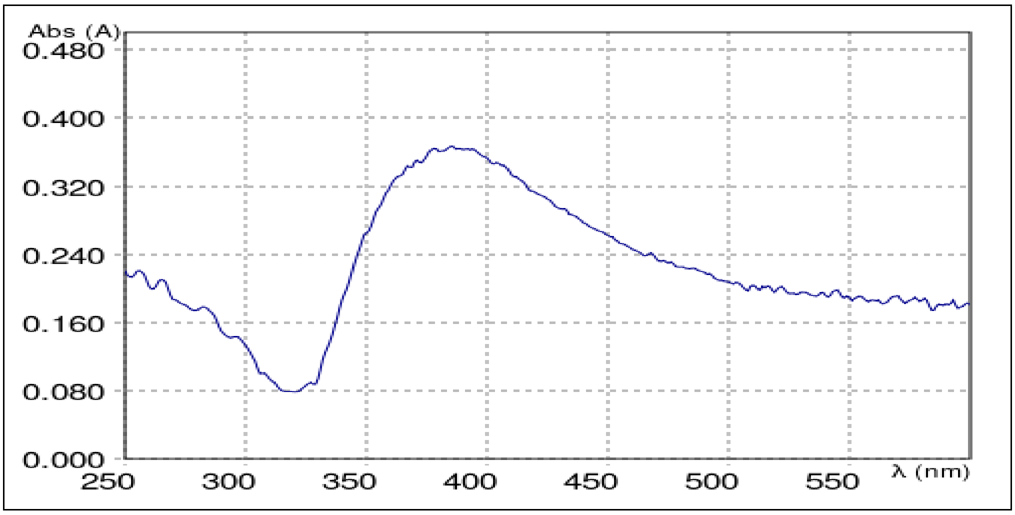

Fig 2: UV absorbance spectrum of AgNPs.

TEM micrograph of the AgNPs.

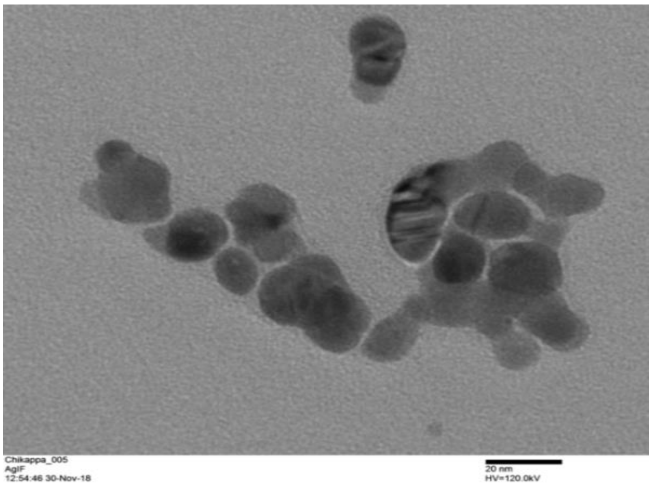

Fig 3: TEM micrograph of the AgNPs.

Size distribution of the AgNPs.

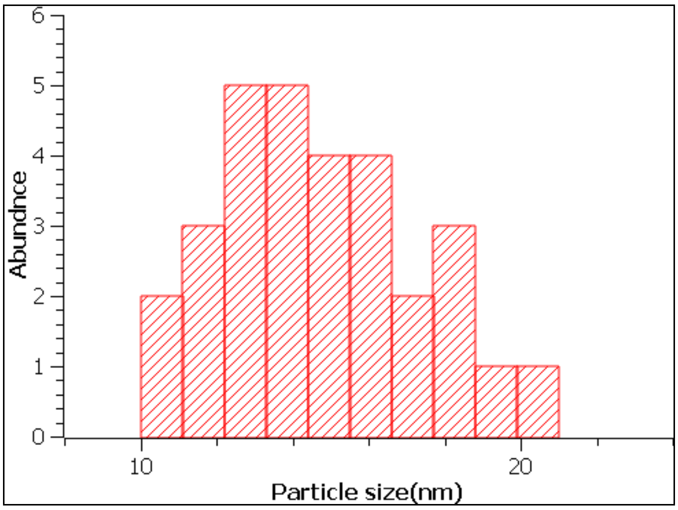

Fig 4: Size distribution of the AgNPs.

Estimation of AgNPs Concentration in Stock solution

The initial concentration of AgNPs in the colloidal suspension is determined by the following method (Kalimuthu Kalishwaralal, *et al.*, 2010) [45]

$$N = \frac{\pi \rho D^3}{6M} N_A$$

N= number of atoms per Ag nanoparticle

ρ = density of FCC silver = 10.5 g/cm3

D = average diameter of nanoparticles estimated using the TEM micrograph

M = atomic mass of silver =107.868g/Mol.

NA = number of atoms per mole= 6.023×10^{23} atoms/Mol.

From the TEM micrograph analysis using ImageJ software, the average diameter of AgNPs found to be 14.748nm i.e., D =14.748nm= 14.748×10^{-7} cm

$$N = \frac{3.14 \times 10.5 \times (14.748 \times 10^{-7})^3}{6 \times 107.868} \times 6.023 \times 10^{23}$$

= 98421 silver atoms per Ag nanoparticle

The concentration of AgNPs, $C = \frac{N_T}{N \times V \times N_A}$

N_T = the number of silver atoms added to AgNO3

V= the volume of reaction solution in L

The concentration of AgNPs, $C = \frac{0.001 \times 6.023 \times 10^{23}}{98421 \times 32 \times 10^{-3} \times 6.023 \times 10^{23}}$

= 3.17×10^{-7} M= 317nM.

This concentration can be considered for further experimental use.

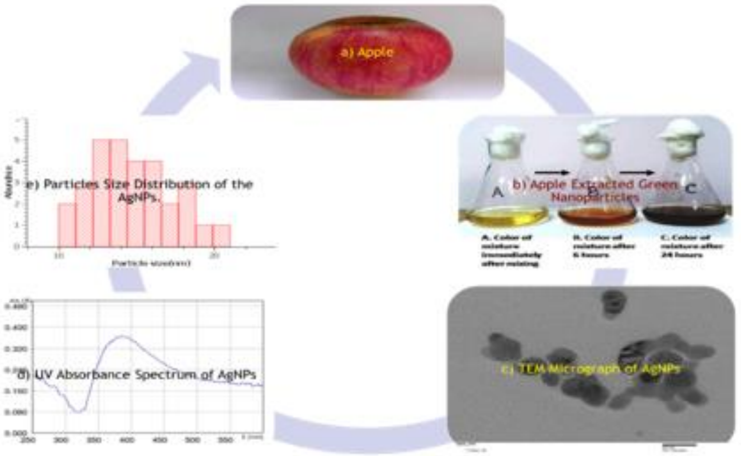

Figure-a. *Fresh apple (Malus domestica)*
Figure-b. *Color change indicates formation of silver nanoparticles*
Figure-c. TEM micrograph of the AgNPs.
Figure-d. UV absorbance spectrum of AgNPs. {*The synthesis of AgNPs is further confirmed by Transmission Electron Microscope (TEM), the JEM-1400 available at Indian Institute of Technology, Delhi. The JEM-1400 Transmission Electron Microscope has a maximum accelerating voltage of 120 KV.*}
Figure-e. Particles Size distribution of the AgNPs.

Fig 5: Flow Chart of the Apple Extracted Green Synthesized Silver Nanoparticles.

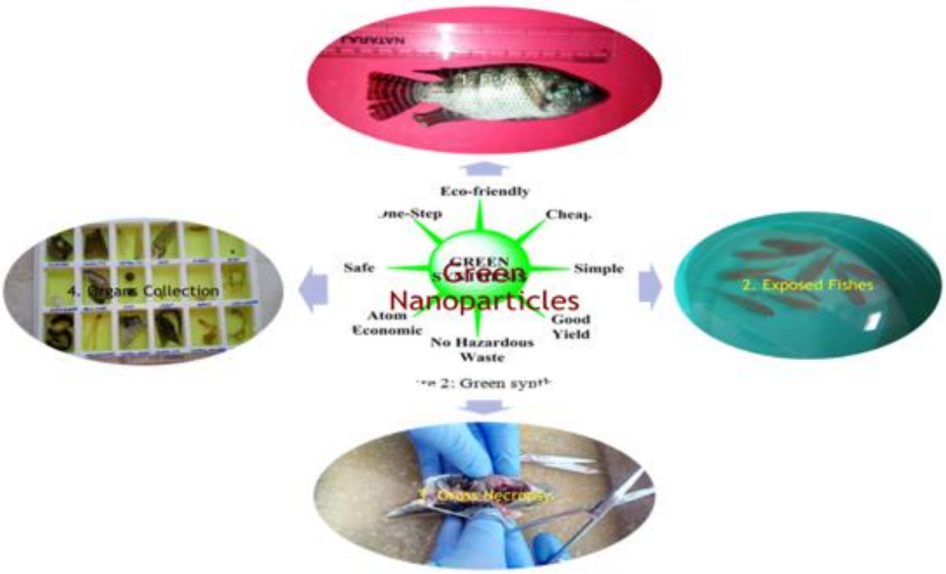

Figure-1. *Oreochromis mossambicus* - Mozambique tilapia (Peters).
Figure-2. Exposed Fishes of Green Synthesized Silver Nanoparticles.
Figure-3. Gross Necropsy of Target Organs.
Figure-4. Green Synthesized Silver Nanoparticles Exposed Fishes Organs Collection.

Fig 6: Flow Chart of the Exposed Fish Tilapia, *Oreochromis mossambicus* (Peters), Shows Apple Extracted Green Synthesized Silver Nanoparticles Non-toxic.

The study which indicates important species used for toxicity testing by name Tilapia, *Oreochromis mossambicus* (Peters) 1852 this is one of the mouth brooding fish in this present globe Figure-1. Which indicates and Figure-2. Shows that the Graph of UV absorbance spectrum of AgNPs and Figure-3. Indicates TEM micrograph of the AgNPs. Figure-4. Size distribution of the AgNPs. Figure-5. Flow chart of the Apple extracted green synthesized silver nanoparticles and Figure-6. Flow chart of the exposed Fish Tilapia, *Oreochromis mossambicus* (Peters), and Shows Apple extracted green synthesized silver nanoparticles is Non-toxic.

Behavioral Toxicological Effects

In this present investigation fishes were exposed to normal tap water with media dose concentration 0ml/L, used as a control group there is no change in all the part of the exposure replicates groups 1-control and 1-treated but we concentrated on both the groups up to the period of 96hrs, it represents and which indicates day-1 250mg/L of green synthesized silver nanoparticles exposed but within 24 hours of time there is no symptoms will be dictated and the body weight, length, behavioural activity will be remains same, there is no morphological anomalies found and finally no gross pathological lesions found and this shows that the fishes are in normal condition. And also the temperature, conductivity, dissolved oxygen and pH in control group by this we understood that there is no maximum fluctuation in physico chemical parameters. And continuation with this in the present investigation fishes were exposed to normal tap water media dose concentration will be 0ml/L, used as a control group and there is no change in all the part of the exposure replicates groups 1-control and 1-treated but we concentrated on both the groups up to the period of 96hrs, and the dose concentration will be 250mg/L fir day-2 of green synthesized silver nanoparticles which dosed but within 48 hours of time there is some symptoms will be dictated behavioural symptoms like, fast movement, settled at the bottom, restlessness, stress, irregular movement. And the body weight, length, behavioural activity will be remains same, there is no morphological anomalies found and finally no gross pathological lesions found and this showed that the fishes are in normal condition and the temperature, conductivity, dissolved oxygen and pH in control group by this we understood that there is no maximum fluctuation in physico chemical parameters.

Discussion

The present study reports the green synthesis of AgNPs using widely available fruit, apple, as its reducing agent. While there have been numerous methods on synthesizing nanoparticles, most of the methods use expensive chemicals and therefore are not cost effective. Moreover, the residues produced are hazardous and toxic. This will result in pollution which could lead to disastrous effects on our environment. Recently the usage of flowery and plant extracts has become an interest due to its clean and simpler approaches. In this present condition of the environment factors shows fluctuating results due to vast and drastic changes. In many countries, the registration of synthetic chemicals involves passing a number of tests for environmental risk assessment and hazard classification. Currently, the hazard assessment of chemicals for fish is based on international standards and guidelines (such as the OECD guidelines) with global toxicity endpoints, such as mortality, growth, reproduction impairment, and biochemical changes in exposed fish. The behavioural abnormalities and state of fishes in both the control and experimental fish was recorded at every hour of first 24 hours.

After introduction of nanao particles, fishes showed interestingly try to jump out of aquarium to avoid the stress followed by increased swimming, restlessness, surfacing and hyper activity. In lower concentration of silver nanoparticles. The fishes showed a slow swimming than the control group. Behavioural manifestations of acute toxicity like erratic swimming, restlessness and surfacing movements were observed in *Cyprinus carpio* exposed to higher concentration of silver nanoparticles over the 24 hours. After 24 hours fishes exhibited lathery and erratic swimming movements suggesting loss of equilibrium at higher concentration of silver nanoparticles. At the time of death evanescent hyper activity was also observed (Dr. Chikkappa Udagani and Dr. Shivaraj Yallappa 2019) [11]. The behavioural abnormalities and state of fishes in both the control and experimental fish was recorded at every hour of first 24 hours. Colloidal silver is one of the most beneficial products of nanotechnology that is effective against many types of pathogenic microorganism, including bacterial fish pathogens (Soltani, M *et al.*, 2009) [46]. Plus (Soltani, M *et al.*, 2011) [47], showed a positive effect of the direct use of silver nanoparticles in reducing fungal infection in trout eggs. However, the commercial application of colloidal silver nanoparticles (AgNPs) to fish is currently lacking clear safety regulations and toxicology data. According to acute toxicity results of published papers, 96 hour LC50 of silver nitrate (AgNO3) for different life stages of rainbow trout ranged from 5.3–20.2 µg/L (Davies, P *et al.*, 1978, Nebeker, A *et al.*, 1983, Buhl, K. and Hamilton S 1991) [10, 34, 8], (Hogstrand C, Galvez F and Wood C, 1996)[16], (Grosell M. *et al.*, 2000) [14]. However based on acute toxicity results of the present study 96 hour LC_{50} of silver nanoparticles (AgNPs) for different life stages of *Cyprinus carpio* ranged from 50ul/L-200ul/L.

Conclusion

The Green synthesis is a simple, low cost and eco-friendly approach without any huge inputs in terms of energy. This is the first report of Green synthesis of silver nanoparticles for this apple extract. Being exhibiting greater antibacterial activity, phytochemical based nanoparticles may stand as a potential remedy in developing drugs against antibiotic resistant bacteria. And this was the first utmost study to expose different species of spreading inconvenience results which produced by the green synthesized silver nanoparticles in nature the fruit apple extracted produced low toxic nanoparticles and the AgNPs were synthesized using apple extract and AgNO3 aqueous solution. The crystalline nature of the AgNPs is evident from sharp peaks in the XRD spectrum, and the average size was 30.25 ± 5.26 nm. The zeta potential value of 5.68 ± 3.28 mV indicates strong agglomeration and precipitation. FTIR analysis suggests that ethylene groups from the apple extract could act as the reducing agent responsible for the reduction of Ag+ into Ag. This method is environmentally friendly, of low cost, and simple and therefore can promote the application of green technology for the production of AgNPs. Our studies have shown that the range from 125 to 1000μg/mL was required to eliminate the Gram-positive and Gram-negative bacteria. E. coli required the lowest MBC which was 125 μg/mL of AgNPs. But the present investigation which shows most of the similar results on fish species and the environmental constrictions are physic-chemical parameters shown good balancing factors which cannot any major changes in morphological and slow behavioural changes which we observed in the high concentration this was the fruit full result when these doses induced to the aquatic species and the patterns of Green synthesis of silver nanoparticles shown the confident results. In this present investigation the direct exposure of freshwater fish *Oreochromis mossambicus* (peters) to the apple extracted colloidal Green synthesis solution AgNPs revealed that the fish were more sensitive to colloidal solution silver nanoparticles during the early life stage of the fish condition and also it impacts on later stages. Furthermore, colloidal silver nanoparticles should be classified as “low-toxic” and “non-toxic” to the embryo stages and juvenile stage of *Oreochromis mossambicus* (peters), respectively, when that the release of Green synthesis of silver nanoparticles into the environment or their direct application as an antifungal/antibacterial behaviour conditions during the hatching period of eggs or larval stages should be avoided. Instead, indirect techniques, like the use of filters containing silver nanoparticles, are better for incubation system water treatment also. Further studies are also recommended on the potential use of a fish in different types of biomarker for toxicity assessments by the Eco-toxicologists evaluated of silver nanoparticles and finally this was the agent which shows "non-toxic" to the entire aquatic ecosystems.

Acknowledgement
I acknowledge with great respect, immense pleasure, and rare privilege of my gratitude to Beloved Parents and My Family Members for their source and force and continuous encouragement during the whole tenure of my academic and research work.

References
1. Sivanesan HK, Kozuch J. Functionalized Ag nanoparticles with tunable optical properties for selective protein analysis, Chemical Communications, 2011; 47(12):3553-3555.
2. Ahmad A, Senapati S, Khan MI, Kumar R, Sastry M. Extracellular biosnythesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete, Thermomonaspora sp. Langmuir. 2003; 19:3550-3553.
3. Ahmad N, Sharma S, Alam MK, Singh VN, Shamsi SF, Mehta BR. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids and Surfaces B: Biointerfaces. 2010; 81:81-86.
4. Anandalakshmi K, Venugobal J, Ramasamy V. Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity, Appl. Nanosci. 2016; 6:399-408.
5. APHA. Standard methods for the examination of water and wastewater. 21st ed. Washington DC, 2005.
6. Wiley B, Herricks T, Sun Y, Xia Y "Polyol synthesis of silver nanoparticles: use of chloride and oxygen to promote the formation of single-crystal, truncated cubes and tetrahedrons," Nano Letters. 2004; 4(9):1733-1739.
7. Bar H, Bhui DK, Sahoo GP, Sarkar P, Pyne S, Misra A. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2009; 348:212-216.
8. Buhl K, Hamilton S. Relative sensitivity of early life stages of *Arctic grayling*, *Coho salmon*, and *Rainbow trout* to nine inorganics. Ecotoxicology and Environmental Safety. 1991; 22:184-197.
9. Philip D. *Mangifera indica* leaf-assisted biosynthesis of well dispersed silver nanoparticles, Spectrochimica Acta Part A: Molecular and Bio molecular Spectroscopy. 2011; 78(1):327-331.
10. Davies P, Goettl J, Sinley J. Toxicity of silver to rainbow trout. Water Research. 1978; 12:113-117.
11. Chikkappa Udagani, Shivaraj Yallappa. Synthesis and Characterization of Colloidal Solution Silver Nanoparticles Effect on Behavioural Changes and LC50 value of fish *Cyprinus carpio* (Linnaeus). IJPBS-International Journal of Pharma and Bio Sciences. 2019; 10(2):68-74.
12. Dwivedi D, Dayashankar, Maheshwar Dubey K. Synthesis, Structural and Optical Characterization of CdS Nanoparticles, Journal of Ovonic Research. 2010; 6(1):57-62.
13. Finney OJ. Toxic effect of profenofos on tissue acety cholinesterase and gill morphology in a euryhaline fish, *Oreochromis mossambicus*. Probit analysis, 2nd edition. Cambridge university press, Cambridge. For Efficient Cellular Delivery, Chemical Engineering Science. 1953; 61:1027-1040.
14. Grosell M, Hogstrand C, Wood C, Hansen H. A nose-to-nose comparison of the physiological effects of exposure to ionic silver versus silver chloride in the European eel (*Anguilla anguilla*) and the *Rainbow trout* (*Oncorhynchus mykiss*). Aquatic Toxicology. 2000; 48(2, 3):327-342.
15. Zhu H, Du M, Zhang M. Facile fabrication of AgNPs/ (PVA/PEI) nanofibers: high electrochemical efficiency and durability for biosensors, Biosensors and Bio electronics. 2013; 49:210-215.
16. Hogstrand C, Galvez F, Wood C. Toxicity, silver accumulation and metallothionein induction in freshwater *Rainbow trout* during exposure to different silver salts. Environmental Toxicology and Chemistry. 1996; 15:1102-1108.
17. Jayshree Annamalai and Thangaraju Nallamuthu. Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency, Appl Nanosci. 2016; 6:259-265.
18. Jiang HS. The effect of chronic silver nanoparticles on aquatic system in microcosms, Environmental Pollution, http: //dx. doi.org/ 10.1016/j. envpol. 01.036. 2017.
19. Caswell KK, Bender CM, Murphy CJ. Seedless, surfactant less wet chemical synthesis of silver Nano wires, Nano Letters, 2003; 3(5):667-669.
20. Liu K, Qu S, Zhang X, Tan F, Wang Z. Improved photovoltaic performance of silicon nanowire/organic hybrid solar cells by incorporating silver nanoparticles, Nanoscale Research Letters, 2013; 8(1):1-6.
21. King Heiden TC, Dengler E, Kao WJ, Heideman W, Peterson RE. Developmental toxicity of low generation PAMAM dendrimers in zebra fish. Toxicol. Appl. Pharmacol, 2007; 225:70-79.
22. Klaueh U, Wagner V, Kelly S, Johnson A, Bryers JD. Efficacy of silver coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. Journal of Biomedical Material Research. 2000; 53:621-631.
23. Kumar A, Vemula PK, Ajayan PM, John G. Silver-nanoparticle embedded antimicrobial plants based on vegetable oil. Journal of Nature Materials. 2008; 7:236-240.
24. Maretti L, Billone PS, Liu Y, Scaiano JC. Facile photochemical synthesis and characterization of highly fluorescent silver nanoparticles, Journal of the American Chemical Society. 2009; 131(39):13972-13980.
25. Li J, Mookerjee B, Wagner J. Purification of melanoma reactive T cell by using a monocyte based solid phase T cell selection system for adoptive therapy. Journal of Immunotherapy. 2008; 3:81-85.
26. Lilius HA. Comparison of the toxicity of 50 reference chemicals to freshly isolated *Rainbow trout hepatocytes* and *Daphnia magnas*. Aquatic toxicol. 1994; 30:47-60.
27. Link S, El-Sayed MA. Optical properties and ultrafast dynamics of metallic nanocrystals, Annu. Rev Phys Chem. 2003; 54:331-366.

28. Malinsky MD, Kelly KL, Schatz GC, Van Duyne RP. Chain length dependence and sensing capabilities of the localized surface Plasmon resonance of silver nanoparticles chemically modified with alkanethiol self-assembled monolayers, Journal of the American Chemical Society, 2001; 123(7):1471-1482.
29. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials, Biotechnology Advances, 2003; 27(1):76-83.
30. Uddin MJ, Chaudhuri B, Pramanik K, Middya TR, Chaudhuri B. Black tea leaf extract derived Ag nanoparticle-PVA composite film: structural and dielectric properties, Materials Science and Engineering B. 2012; 177(20):1741-1747.
31. Madanagopal Nalini, Mahalingam Lena, Palanisamy Sumathi, Chandran Sundaravadivelan. Effect of phyto-synthesized silver nanoparticles on developmental stages of malaria vector, *Anopheles stephensi* and dengue vector, *Aedes aegypti*, Egyptian Journal of Basic and Applied Sciences. 2017; 4:212-218.
32. Muhammad Saleem. Toxicity of silver nanoparticles in fish: a critical review, Journal of Biodiversity and Environmental Sciences (JBES) ISSN: 2220-6663 (Print) 2222-3045 (Online). 2015; 6(5):211-227.
33. Basavegowda N, Idhayadhulla A, Lee YR. Tyrosinase inhibitory activity of silver nanoparticles treated with *Hovenia dulcis* fruit extract: an *in-vitro* study, Materials Letters, 2014; 129:28-30.
34. Nebeker A, McAuliffe C, Mshar R, Stevens D. Toxicity of silver to steelhead and *Rainbow trout*, *Fathead minnows* and *Daphnia* Environmental Toxicology and Chemistry. 1983; 2:95-104.
35. Noginov MA, Zhu G, Bahoura M, Adegoke J, Small C, Ritzo BA *et al*. Enhancement of surface plasmons in an Ag aggregate by optical gain in a dielectric medium, Opt Lett; 2006; 31(20):3022-3024.
36. OECD. Organization for Economic Co-operation and Development, TG 203 OECD Guideline for testing of chemicals (Fish, Acute Toxicity Test), 1992.
37. Khanna PK, Singh N, Charan S, Subbarao VVVS, Gokhale R, Mulik UP. Synthesis and characterization of Ag/ PVA Nano composite by chemical reduction method, Materials Chemistry and Physics. 2005; 93(1):117-121.
38. Zhang P, Shao C, Zhang Z. In situ assembly of well dispersed Ag nanoparticles (AgNPs) on electro spun carbon Nano fibers (CNFs) for catalytic reduction of 4-nitrophenol, Nano scale, 2011; 3(8):3357-3363.
39. Mendoza-Res´endez R, N´ u˜nez NO, Barriga-Castro ED, Luna C. Synthesis of metallic silver nanoparticles and silver organometallic nanodisks mediated by extracts of *Capsicum annuumvar*. Aviculare (piquin) fruits," RSC Advances. 2013; 3(43):20765-20771.
40. Rao CN, Cheetham AK. Science and technology of nanomaterials current. 2001.
41. Sukumaran Prabhu, Eldho K Poulose. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects, e International Nano Letters, 2012.
42. Ismail WA, Ali ZA, Puteh R. Transparent Nano crystallite silver for anti-bacterial coating, Journal of Nanomaterial's, vol. 2013, Article ID 901452, 2013, 6.
43. Xu ZP, Zeng QH, Lu GQ, Yu AB. Inorganic Nanoparticles as Carriers, 2006.
44. Yin Y, Li Z-Y, Zhong Z, Gates B, Xia Y, Venkateswaran S. Synthesis and characterization of stable aqueous dispersions of silver nanoparticles through the Tollens process, Journal of Materials Chemistry, 2002; 12(3):522-527.
45. Kalishwaralal K, Barath Mani Kanth S, Pandian SRK, Deepak V, Gurunathan S. Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Colloids Surfaces B Biointerfaces. 2010; 79(2):340-4.
46. Soltani M, Ghodratnema M, Ahari H, Ebrahimzadeh Mousavi HA, Atee M *et al*. The inhibitory effect of silver nanoparticles on the bacterial fish pathogens, *Streptococcus iniae*, *Lactococcus garvieae*, *Yersinia ruckeri* and *Aeromonas hydrophila*. Int J Vet Res. 2009; 3(2):137-42.
47. Soltani M, Esfandiary M, Sajadi MM, Khazraeenia S, Bahonar ARAH. Effect of nanosilver particles on hatchability of rainbow trout (*Oncorhynchus mykiss*) egg and survival of the produced larvae. Iran J Fish Sci. 2011; 10(1):167-76.