Nanotoxicity and Accumulation, Biochemical Parameters of Green Synthesized Iron Oxide Nanoparticles on Platy Xiphophorus Maculates

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Research Article

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Abstract

The purpose of this research was to develop an ecological risk based on effects of nano based toxicity on fish species posed by nanoparticles. Hardly few studies have reported the toxicity on *Xiphophorus maculates.* Therefore, the aim of this research is acute toxicity, accumulation and biochemical parameters (protein, carbohydrates and lipids) of green synthesized iron oxide nanoparticles on *Xiphophorus maculates.* Synthesized iron oxide nanoparticles were characterized by UV-Visible spectrophotometer, Scanning Electron Microscope, Energy Dispersive X-Ray Spectroscopy, Fourier Transform Infrared Spectroscopy and X-Ray Diffraction. LC_{50} (0, 10, 100, 500, 750 and 1000 ppm) of green synthesized iron oxide nanoparticles for 96 hrs and sub-lethal studies, platy was exposed to different concentrations (0, 100, 200, 300 and 400 ppm) of biosynthesized iron oxide nanoparticles for 7 days. Accumulation of iron oxide nanoparticles in gills increased based on concentration and biochemical parameters (protein, carbohydrate and lipids) in muscle and gill platy were gradually abated with increased concentration of iron oxide nanoparticles. In conclusion, both acute and sub-lethal toxicity of green synthesized iron oxide nanoparticles more pronounced at higher concentration with two different periods. This is the first report on green synthesized iron oxide nanoparticles toxicity, accumulation and biochemical parameters using *Xiphophorus maculates* as a bioindicators.

Introduction

Nanotoxicity is an main aspect of nanotechnology that deals with toxicological effects of synthesized nanoparticles on aquatic organisms. An aquatic environment is most vulnerable to contamination from synthesized nanoparticles and its toxicity of these nanoparticles is very limited [1–3]. Especially, fishes are exposed exposed to nanoparticles in three primary ways. Dermal, inhalation, direct absorption through the skin by swimming in polluted waters, by direct uptake of nanoparticles through the gills during respiration and water circulation. Gills are linear connection with polluted water from the aquatic biota. Hence, there is a urgent need on nanotoxicological clear view for the sustainable development of nanotechnology [4].

In recent years, physical, chemical and biological methods are developed for the synthesis of metal oxide nanoparticles. Also, several methods have been reported for the synthesis of iron oxide nanoparticles, such as hydrolysis, thermal decomposition, chemical co-precipitation and sol-gel method [5]. Among them, the biosynthesis of nanoparticles has received much attention and convenient biological materials namely fungi, bacteria, biomolecules and plant extracts are widely used to replace chemical and physical methods [6]. Plant extracts are quickly reducing the metal ions when compared to micro-organisms. Moreover, biosynthesis of nanoparticles using plant extracts has been considered a more environmentally safer and more cost- effective alternative to chemical and physical methods[7]. The main advantage of biosynthesis is to control the particle size, shape and physicochemical properties. Also, biomolecules of plants can act as capping and reducing agents and it increases the percentage of reduction rate and stabilization of nanoparticles [8].

The average amount of nanoparticles released into the aquatic environment per year is 65 tons come from manufactured goods, effluent and spillage during the shipping and handling of products or through deliberate disposal. Nanoparticles may contaminate the aquatic environment and exert toxic effects on aquatic species including fishes [9]. The Xiphophorus maculatus, popularly known as platyfish, is a good model fish for ecotoxicological bioassays [10–14]. The work related to the toxicity of green synthesized iron oxide nanoparticles using *Casia fistula* leaf extract, accumulation in gills and biochemical parameters of platy *Xiphophorus maculatus* is totally wanting in this current era.

Materials And Methods

Synthesis of iron oxide nanoparticles:

Cassia fistula is a medium-sized deciduous tree of tropical and subtropical areas, widely showing a large number of medicinal properties and extracts of leaves that have significant antibacterial activity and other valuable pharmacological activities. For this Co-precipitation method was used for the synthesis of iron oxide nanoparticles prepared *Casia fistula* leaf extract was used in order to reduce and cap the Fe ions. 5ml of leaves of methanol extract (prepared by Soxhlet method) were taken (pH 6) and added 0.1M $FeCl_2$ and $FeCl_3$ (1:2) and continuously stirred in a magnetic stirrer at 80 ± 1°C for sixty minutes. The solution pH was adjusted to 11 by adding ammonia and the synthesis of nanoparticles were confirmed by colour changes from greenish-yellow to dark greenish black precipitate. The mixture was transferred into an autoclave for two days and maintained at 180°C for twelve hours in a hot air oven and cooled at room temperature, the precipitate was washed several times with double-distilled water and absolute ethanol and separated by centrifugation. The obtained samples were collected and dried in an oven at 80°C for overnight.

Characterization of iron oxide nanoparticles :

The absorbance spectra of samples were measured in wavelength within the range from 200-800 nm using a JASCO-V-530-UV-VIS Spectrophotometer. The samples were scanned between the angles 0° to 90° to obtain the equatorial reflection. The morphology of the material was studied using Tesco SEM-VEGA III lmu – a type of Scanning Electron Microscopy operating at 20kv in the vacuum on powder samples and the chemical composition of iron oxide nanoparticles were analyzed by employing Energy Dispersive X-ray Spectrometer. Measure the presence of functional groups of biomolecules which is responsible for the synthesis of iron oxide nanoparticles were recorded by thermo-scientific NICOLETTIIS5 over the spectral range of 4000-400 cm^{-1}. The structure and crystalline size of nanoparticles were determined by XRD using SHIMADZU (Model XRD 6000) with nickel-filtered Cu Kα radiation in the 2θ range (λ = 1.5418Å) from an X-ray tube run at 40 kV and 30ma.

Toxicant preparation:

For toxicity studies, Platy *Xiphophorus maculates* fingerlings(3.5 ±1.5g) were collected from A.M Fish Farm, Madurai, Tamil Nadu, India and transported to the laboratory in polythene bags filled with oxygenated water. Fishes were acclimated in round plastic troughs for a period of 15 daysat28 ± 2^{o}C. Before introducing the platy fish, basic water quality parameters such as pH, Temperature, dissolved oxygen, dissolved carbon dioxide, hardness, alkalinity, and Chloride were estimated [15]. 5 healthy platy fish, with an average length of 4±1 cm and an average weight of 4-5 ±1 g were selected and introduced into each trough. The behaviour and survival of fish were observed in each concentration for a period of 7days. Different concentrations (0, 10, 100, 500, 750 and 1000 ppm) of the biosynthesized iron oxide nanoparticles were taken and observed the survival studies were for 96hrs. For sub-lethal studies, platy was exposed to different concentrations (0, 100, 200, 300 and 400 ppm) of biosynthesized iron oxide nanoparticles for 7 days.

Accumulation and biochemical parameters of iron oxide nanoparticles:

Fish were analyzed for accumulated iron-on gill surfaces using a semi-quantitative filterpaper technique [16]. Filter paper discs (Whatman No.1) of 13 mm diameter were placed between the first and second-gill hemibranchs on the left side (dorsalview) of test and control fish and left for 30 seconds. Discs were then placed in 10 ml 1% HCl and shaken vigorously for 5 seconds. Samples were then left at 4^{o} C over night and stored at 20^{o}C until required for analysis. After thawing, filter discs were removed and solutions analyzed for iron by AAS (Perkin Elmer A Analyst 100). Results were calculated as mg Fe kg^{-}1 dry weight. During the sub-lethal tests, fish were fed with pellets that may have contained iron, however, the iron content of control exposure water remained close to detect ion limits of the AAS instrument. Biochemical parameters such as protein, carbohydrate, and lipid [17-19] in the muscle, and gill of Platy fish was estimated. Fish used in the present research was in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA, Ministry of Environment & Forests(Animal Division), Government of India] on the care and use of animals in scientific research and also approved by the Institutional Ethical Committee for Research on Human and Animal Subject (IECRHAS) from The Gandhigram Rural Institute- Deemed to be University, Govt. of India, Gandhigram, Tamil Nadu, India.

Results

Uv absorbance spectra of Fe_3O_4 nanoparticles were absorption at 371.79 nm (Fig. 1). This peak was occurred due to precipitated Fe_3O_4 ions on the surface of nanoparticles. Scanning Electron Microscopy indicates the spherical and rectangular shape of synthesized nanoparticles (Fig. 2a, b & c). SEM results indicated that the iron oxide nanoparticles are spherical and rectangular in shape. The element composition of the synthesized iron oxide nanoparticles was identified by an energy-dispersive X-ray spectroscopy system shows three peaks located between the 0.5 KeV and 6.5 KeV (Fig. 3). The maximum peak is located on the spectrum at 6.5 KeV from Fe. These maximum peak located on the spectrum at 0.5 KeV clearly indicates oxygen. Fourier Transform Infrared Spectroscopy measurements were carried out to

identify the possible functional groups responsible for the reduction of the Fe ions in biosynthesized iron oxide nanoparticles. The FTIR spectrum of iron oxide nanoparticles were analyzed in the range of 4000-400cm^{-1} (Fig. 4). The bands observed at 3437.05, 2918.88, 1714.61, 1628.25 and 543.93 are associated with functional groups of alcohol, phenols, primary amines, ketones and O-H, C-H, C-Br stretching of proteins. The structure and crystalline size of nanoparticles were determined by the XRD diffraction peaks and are indexed as 10.75^{0} (020), 24.12^{o} (022), 33.14^{o} (110), 35.62^{o} (111), 40.87^{o} (041), 49.47^{o} (104), 54.02^{o} (124) and 62.45^{o} (125) (Fig. 5).The synthesized iron oxide nanoparticles was confirmed by XRD and the average size of the crystal is 20 ± 14. Water quality parameters of tap water such as pH, temperature, dissolved oxygen, dissolved carbon dioxide, hardness, alkalinity and chloride has been analyzed before introducing platy fish to iron oxide nanoparticles dissolved in water (Table 1). The basic observation is exposed to their on oxide nanoparticles is presented in Table 2. Survival Studies of Platy Fish exposed to Iron oxide Nanoparticles is presented in Table 3. Iron accumulation on platy fish gills was gradually increased by 100 to 400 ppm when compared to the control (Table 4). Total protein, carbohydrate and lipid in muscle and gill of Platy fish decreased with increased concentration of iron oxide nanoparticles (Table 5).

Discussion

UV-Vis spectrum shows that the maximum absorbance of synthesized iron oxide nanoparticles peaks at 371.79 nm. UV visible spectroscopy analysis showed a peak at 277 nm for the synthesis of iron oxide nanoparticles using *Jetropha gossypifolia* leaves extract [20]. SEM results indicated that the iron oxide nanoparticles are spherical and rectangular in shape. Green synthesized of iron oxide nanoparticles were spherical in shape [21 & 22]. The same results were reported using *Azadirachta indica* (Neem) leaf powder as a biosorbent for the synthesis of iron oxide nanoparticles [23]. In the present study, the elemental composition of iron oxide nanoparticles is iron and oxygen. A similar result has reported the synthesis of iron oxide nanoparticles using Eucalyptus globulus leaf extract [24]. Also reported that the elemental composition of iron oxide nanoparticles was Iron, Oxygen, Calcium and Chloride [25]. The FTIR spectrum of iron oxide nanoparticles were analyzed in the range of 4000-400cm^{-1}.The FT- IR spectrum of iron oxide nanoparticles has a conformation peak at 511 to 535 cm-1 [26]. Also reported that the main functional groups of iron oxide nanoparticles are alcohol, phenols and a primary amine [27]. XRD shows that the synthesized iron oxide nanoparticles were crystal in nature and the average size is 20 ± 24. Also reported that the synthesized iron oxide nanoparticles were crystal nature confirmed by XRD and the nano-crystal average size is 10 to 16nm [28]. A similar XRD result was also reported by using *Withania sominefera* leaf extract [4].

In the present study water quality parameters were studied before introducing the fish. Similar water quality parameters were reported (temperature- 28.5, pH- 6.5, dissolved oxygen – 5.5 to 6.5 mg/l, nitrate – 0.4 to 0.6 ppm, salinity 25 ppt [30]. Also reported that the mean values of physicochemical parameters of water samples such as temperature, pH, dissolved oxygen (DO), total alkalinity and total hardness are 27ºC, 7.7, 7.00mg/L, 202mg/L and 51.6mg/L, respectively [31]. The behavior response of platy fish such

as aggressive and jerking movement was noted among some fishes. Behavioural changes are the most sensitive indication of potential toxic effects [32]. Behavioral alternations like erratic swimming, restlessness and surfacing may be an avoiding reaction to heavy metal narcotic effects or to change in sensitivity of chemo-receptors [33]. Abnormal behaviour of *Labeo rohita* during the experimental period when treated with iron oxide nanoparticles [34]. In this study, platy fish was treated to various lethal concentrations of iron oxide nanoparticles during the period of 4 days (96 hr) and mortality was observed. Sub-lethal concentration was carried for 7 days in which no mortality was observed. The mortality / survival of fish in iron oxide treated was recorded after 96 hours and the concentration at which 25% mortality of fish occurred was taken as the median lethal concentration [36]. 100% mortality was observed in 6 hours, under the influence of different concentrations of iron oxide nanoparticles and the toxic effect was observed on hematological parameters [36]. The median lethal concentration (LC_{50}) of chemically produced nanoparticles was estimated as 0.055 mg/L during 48 hours of exposure [37]. The accumulation of metals on fish gills is in direct contact with contaminant water and it causes the gill damage respiratory route [38 – 4]. The increased iron concentration on gills is due to hydrolysis and polymerization in agreement with the fish body [39]. Total protein, carbohydrate and lipid in muscle and gill of Platy fish decreased with increased concentration of iron oxide nanoparticles. A similar decrease was reported in Zebrafish *Danio rerio* [40]. Green synthesized silver nanoparticles exposed on *Mystus gulio's* muscle and gills, the protein was decreased for a period of 15 days [41]. Total protein, carbohydrate and lipid were decreased in Fe3O4 nanoparticles treated *Labeo rohita* [42].

Conclusions

Iron oxide nanoparticles were biosynthesized by using *Casia fistula* leaf extract as reducing agent. Biosynthesized iron oxide nanoparticles were characterized using UV-Vis, SEM, EDAX, FT-IR and XRD. The quantity of Iron oxide nanoparticles was accumulated in the gill of platy was in relation to the concentration of iron oxide nanoparticles. Protein, carbohydrate and lipid content in muscle and gill of platy decreased with increased concentration of iron oxide nanoparticles. In this toxicity may lead the high accumulation in the short duration of 7 days. This findings strongly suggested giving a alarm to the Pharmacuetical related application.

Declarations

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Conflict of Interest

Authors declare no conflict of interest.

References

1. Saravanan, R. Suganya, M. Ramesh, R.K. Poopal, N. Gopalan, N. Ponpandian, J. Nanopart. Res. 274: 1(2015).
2. Canesi, C. Ciacci, D. Vallotto, G. Gallo, A. Marcomini, G. Pojana, Aquat. Toxicol. 96, 151 (2010).
3. M. Scown, R. Van Aerle, C.R. Tyler, Crit. Rev. Toxicol. 40:653 (2010).
4. Suganya, C.M. Ramakritinan, M.R. Rajan, J. Inorgan.Organomet. Poly. Mat. 28(6):2603–2611 (2018).
5. Petcharoen, A. Sirivat, Mater. Sci. Engi. B. 177(5):421 – 427(2012.).
6. Baskaran, A. Varuvel, G. Vigila, T. Parimelazhagan. D. Muralidhararao, S. Zhang, Int. J.Nanomed.11: 5789 – 5806 (2016).
7. Rai, A. Yadav A. Gade, Crit. Reviews Biotech. 28 (4): 277 – 284 (2008).
8. Latha, M. Gowri, Int. J. Sci. Res. 3 (11):1551 – 1556 (2014).
9. Farre, K. Gajda–Schrantz, L. Kantiani, D. Baecelob, Bioanalyt. Chem. 393:81-95(2009).
10. L. Kinnberg, B. Korsgaard, P. Bjerregaard, A. Jespersen, J. Exp. Biol. 203, 171–181 (2000a).
11. Kinnberg, B. Korsgaard, P. Bjerregaard, Mar. Environ. Res. 50, 169–173(2000b).
12. Tarkhani, M.R. Imanpoor, World J. Fish Mar. Sci. 4, 512–516 (2012). Sadeghi, M.R. Imanpoor, Iran.J.Toxicol. 9, 1278–11276 (2015).
13. R. Tancredo, J.V. Ferrarezi, N.C. Marchiori, M.L. Martins, Aquac. Int. 27, 685–694 (2019).
14. da Silva Souza, D. Lacerda, L.L. Aguiar, M.N.C. Martins, Ecol. Indic., 111:105980 (2020).
15. , AWWA., WEF., Standard Methods for the Examination of Water and Wastewater (2012).
16. C. Playle, C.M.Wood, J.Fish.Bio.38:791-805 (1991).
17. H. Lowry, N.J. Rosenbrough, A.L. Farr, J. Biol. Chem. 193(1):265–275 (1951).
18. V. Carrol, R.W.Longley, J.H.Roe, J. Biol. Chem. 220(2): 583–593 (1956).
19. Barnes, J. Blackstock, J. Exp.Mor, Bio. Ecol.12:103-118 (1973).
20. Karkuzhali. A. Yogamoorthi, Int. J. Nano. Sci. Nanotech. 6(1):47 – 55 (2015).
21. Santhoshi, A. ShakilaBanu, G.A. Kurian, Int. J. Phar. Pharmaceut. Sci.7(1):75-80 (2015).
22. Eshaghi, F. Vafaeinezhad, S. Hooshmand, Pro.Saf.Envir. Prot.102: 403 – 409(2016).
23. P. Devatha, K . Jagadeesh, M. Patil, Environ. Nanotech. Monit. Manage.9 (5):85 – 94 (2018).
24. Balamurugan, S. Saravanan, T. Soga, Surface Sci.Nanotech.12:363-367 (2014).
25. Eric C. Njagi, Hui Huang, Lisa Stafford, Homer Genuino, Hugo M. Galindo,John B. Collins, George E. Hoag, Steven L. Suib., Lang. 27:264–271 (2011).
26. EL-Kassas Hala, A. Aly- Eldeen Mohamed, M. Gharib Samiha, Nat. Inst. Oceanogra. Fish. 35(8):89 – 98 (2015).
27. Arokiyaraj, M. Saravanan, N.K. Udaya Prakash, M. Valan Arasu, B. Vijayakumar, S.Vincent, Mat. Res. Bull. 48:3323–3327 (2013).
28. R. Rajan. M. Manjula Devi, D. Suganya, Int. J. Phar. Sci. Nanot. 14(5):5603-5611 (2021).

29. Suganya, M.R. Rajan, R. Ramesh, Int. J.Curr. Res. 8(11):42081-42085 (2016).
30. Mohan Raj, A.R. Thrunavukkarasu, M. Kailasam, M. Muralidha, R. Subburaj, P. Stalin, Ind. J. Sci. Tech. 6 (4):4330-4335 (2013).
31. Shahzad, M. Naeem Khan, F. Jabeen, N. Kosour, A. Shakoor Chaudhry, M. Sohail, N. Ahmad, Int. J. Environ. Sci. Pollut. 25(16):15943-15953 (2018).
32. Chebbi, H. Gang, M. David, Sci. Asia. 36:12-17 (2010).
33. Maruthanayagam, G. Sharmila, A .Kumar, Ecol.Ethol.Aqu. Biotech. 31:119-127 (2002).
34. Keerthika, R. Ramesh, and M.R. Rajan, Nanotoxi. 5(8):158-160 (2016).
35. J.Thorp, P.S. Lake, Aust. J. Mar. Water Res. 25:97–104 (1974). Kosyan, Kan R. Kantor., Ruthenica 26 (2) : 85 – 121 (2016).
36. A.Johari, M.R. Kalbassi, M. Soltani, I.J.Yu, IranJ.Fish. Sci. 2(1):76-95 (2012).
37. J. Bebianno, F. Geret, P. Hoarau, M.A. Serafim, M.R. Coelho, Biomark. 9(4-5):305-330 (2004).
38. C. Teien, O.A. Garmo, A. Atland, B. Salbu,Environ. Sci. Tech. 42 (5):1780 – 1786 (2008).
39. Tamilmathi, M.R.Rajan, Nat. Environ. Poll. Tech. 20(1):211-219 (2021).
40. Abirami, A.G.R. Jose, B. Govindarajulu, J. Karthikeyan. Int. J. Phar. Pharmaceuti. Sci. 9(11):192-198 (2017).
41. Keerthika, R. Ramesh, M.R.Rajan, Inter. J. Fish. Aqua. Stu.;5(4):01-06 (2017).

Tables

TABLE 1. FT-IR FUNCTIONAL GROUP REPRESENTATION OF IRON OXIDE NANOPARTICLES

Bands	Functional group	Type of vibration	Intensity
3437.05	Alcohols, phenols	O-H stretch, H-bonded	Strong, broad
2918.88	Alkanes	C-H stretch	Medium
1714.61	Ketones, saturated aliphatic	C=O stretch	Strong
1628.25	Primary amines	N-H bond	Medium
543.93	Alkyl halides	C-Br stretch	Medium

TABLE 2. COMPOSITION OF SUPPLY WATER USED DURING ACCLIMATION AND EXPOSURE WITH IRON OXIDE NAOPARTICLES

S.No	Parameters	Results
1	pH	7.6 (7.0-7.7)
2	Dissolved oxygen	6.52 mg/l
3	Dissolved CO2	6.2 mg/l
4	Hardness	120.7 mg/l
6	Chloride	184.6 mg/l

TABLE 3. BASIC OBSERVATION OF PLATY FISH EXPOSED TO IRON OXIDE NANOPARTICLES

S.No	Activity	OBSERVATION
1	Circular swimming	Yes
2	Jerk movement	Yes
3	Bottom resting	No
4	Surface respiration	Yes
5	Aggressive movement	Yes
6	Excess of mucous secretion	No
7	Mortality observation	Yes
8	Behavior observation	Yes
9	Breathing movement	Surface

TABLE 4. SURVIVAL STUDIES OF PLATY FISH EXPOSED TO IRON OXIDE NANOPARTICLES

CONCENTRATION (ppm)	EXPOSURE DURATION (Hours) 24 hrs	48 hrs	72 hrs	96 hrs
Control	NM	NM	NM	NM
10 ppm	NM	NM	NM	NM
100 ppm	NM	NM	NM	NM
500 ppm	NM	NM	3	2
750 ppm	3	2	_	_
1000 ppm	5	_	_	_

NM – No Mortality

Table 5. Accumulation of Iron Oxide Nanoparticles in Gills of Platy Fish

Concentration	Iron Oxide Nanoparticles (g/mg^{-1})
Control	144.01
100	472.80
200	949.20
300	1432.99
400	1916.40

Figures

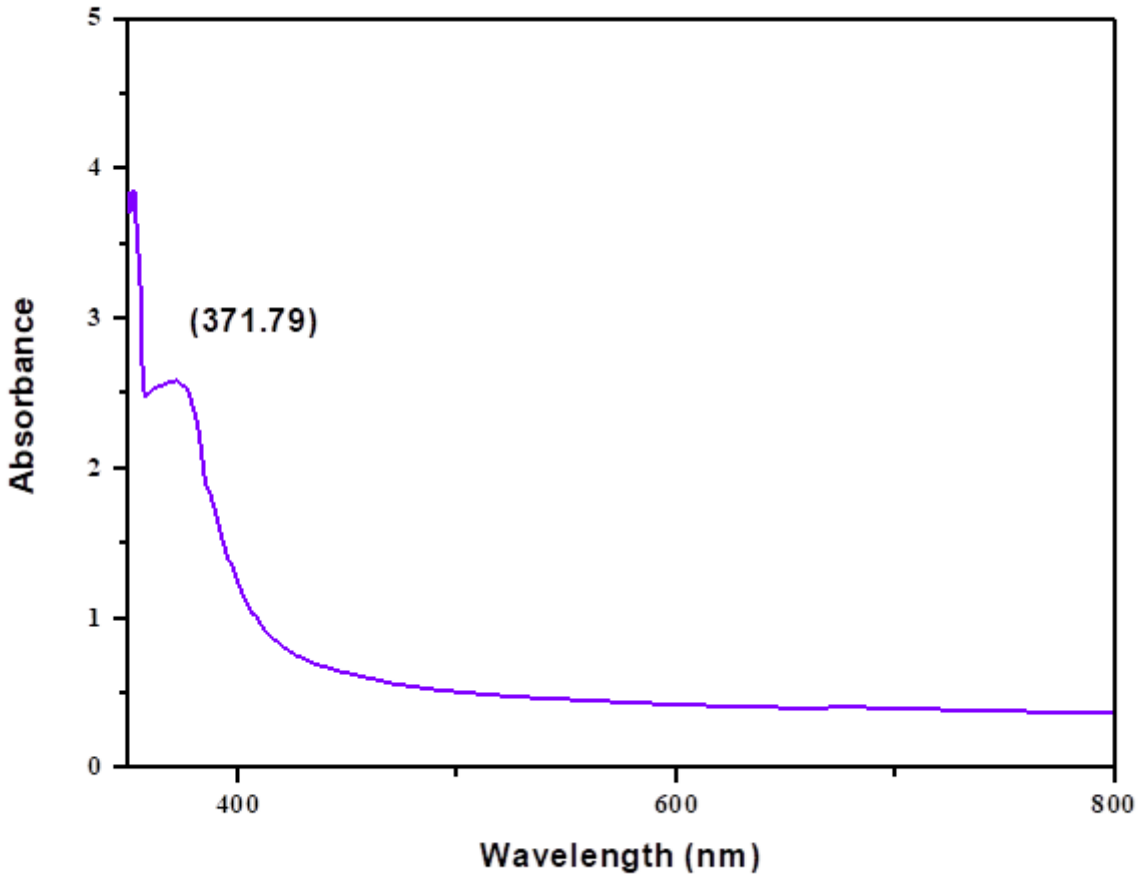

Figure 1

UV-VIS IMAGE OF IRON OXIDE NANOPARTICLES

Fig 2(a)

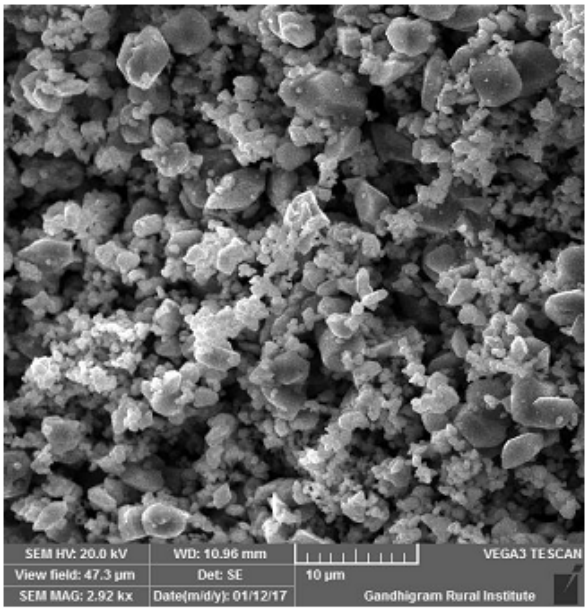

Fig 2(b)

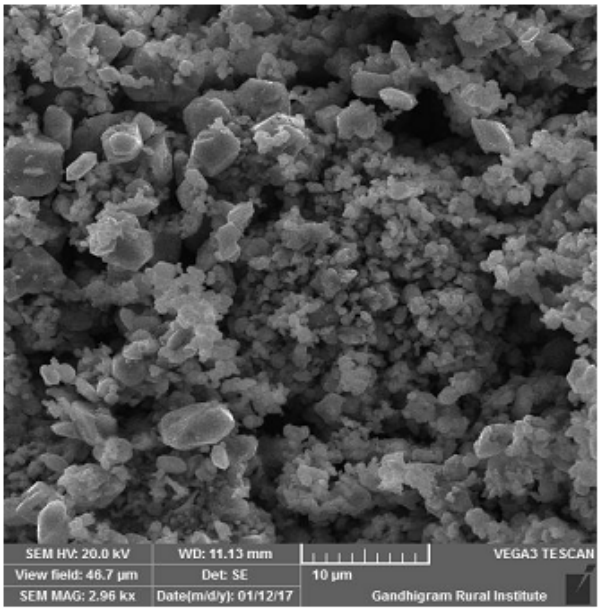

Fig 2(c)

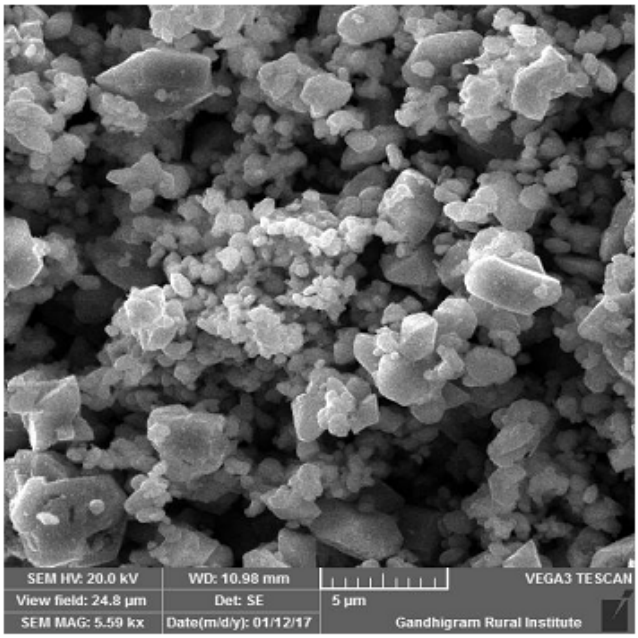

Figure 2

SEM IMAGE OF IRON OXIDE NANOPARTICLE

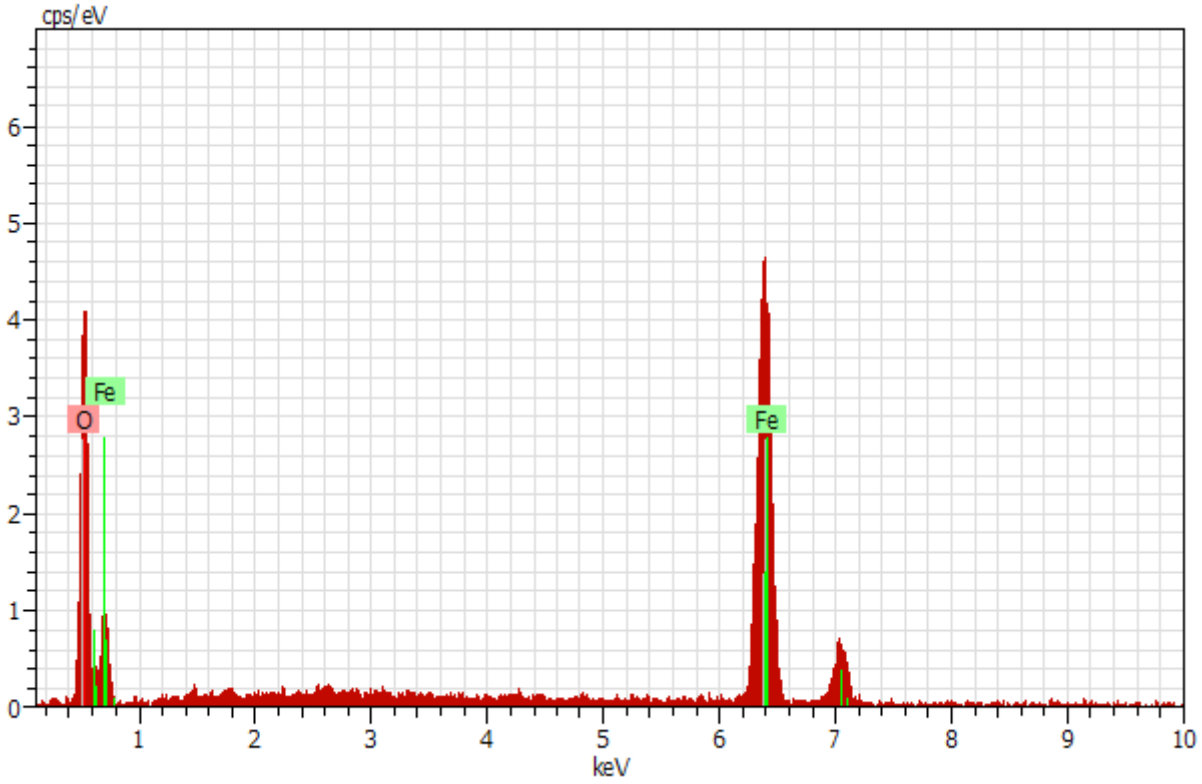

Figure 3

EDAX IMAGE OF IRON OXIDE NANOPARTICLES

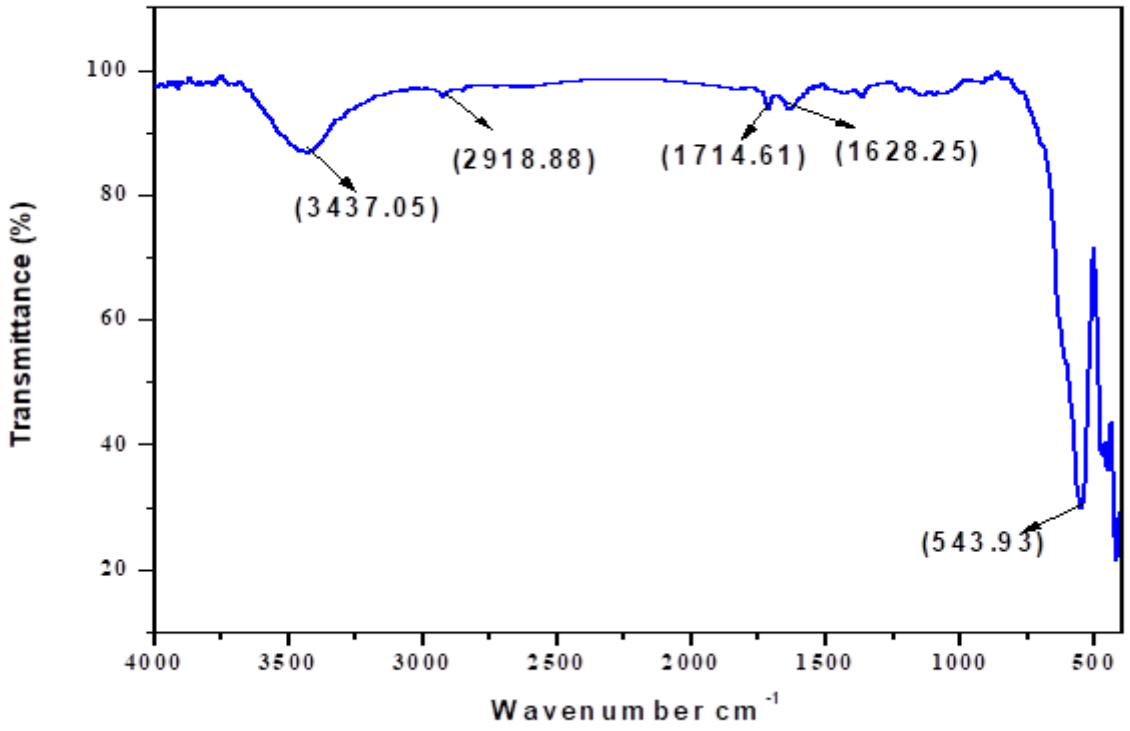

Figure 4

FT-IR IMAGE OF IRON OXIDE NANOPARTICLES