Nanoparticles of magnesium oxide with an anthelmintic activity produced by a green principle

M.Kavitha[1], G. Singaravelu[2*], M.Nagalingam[2], S. Rajeshkumar[3]
[1]Thiruvalluvar University, Vellore 632 115, Tamil Nadu, India
[2]*Thiruvalluvar University, Vellore 632 115, Tamil Nadu, India
[3]PG and Research, Indo-American College, Cheyyar - 604407, Thiruvannamalai, Tamilnadu, India
[4]Nanobiomedicine lab, SaveethaDental College and Hospital, SIMATS, Chennai – 600077, Tamilnadu, India

Abstract
Nanoscience is a burgeoning transdisciplinary field of research leading to revolution in wide field of science. Interplay of phytochemicals and nanomaterials presents an exciting horizon for synthesizing novel nanomaterials. Herein we addressed green principle mediated synthesis of magnesium oxide nanoparticle (MgO) and their anthelmintic functionality. Newly found MgO nanoparticles was characterized and their in-vitro studies. Transmission electron microscopy analysis confirmed the rod shape of MgO nanoparticles with an average particle size 89.8nm. It was recorded that the MgO nanoparticles exhibited significant vermifugal potential and demonstrated paralysis as well as the death of the worm in a time comparable to standard drugs with least concentration.

Introduction

Nanotechnology, as a burgeoning transdisciplinary field of research in aarray of fields, has the potential to enable advancements in applications[1.]Currently, due to their unusual exceptional physicochemical functional high surface-to-volume ratio, and exclusivenanosize structural features, metal oxide nanomaterials, and hybrid nanomaterialsare being increasingly applied as an alternative to conventualagents in life science applications.

Currently, researchers have demonstrated novel products that are nanosized to breakthrough towards exclusive and tunablefeatures of the applied materials. An important issue of nanotechnology is related to the design of experimentalprotocols for the synthesis of nanoparticles (NPs) with different physicochemical features. Recently, researchers have attempted to find biogenic routes for the synthesis of nanoparticles that will act as alternatives tophysicalorchemicalprotocols. Biological synthesis of nanoparticles are considered safe and environmentalbenign, cost-effective and ensure the complete expulsion of toxic chemicals. About from that, the synthesis of nanoparticles using biological resources particles in green chemistry is biocompatible, since functional biomolecules which actively reduce metal ions. Magnesium oxide nanoparticlesaresignificant materials find application as catalysts, electronic and photonic devices. MgO particles are inorganic materials with wide band gaps. It has, high melting point, low heat capacity and is highly functional. Accordingly, it has been utilized for insulation applications in wide areas [2,3].

Magnesium oxide nanoparticles are reported to have potent antibacterial activity with the leverage of being non-toxic. MgONPs have been identified as safe materials by the United States Food and Drug authorities. Recent researchhas led to distinct developments with wide potential in life science[4]. It has been demonstrated that, MgONPs can relieve heartburn, initiate post-activation of bone repair scaffolds and act as hyperthermia material in cancer treatment[5].

An important area of nanotechnology is related to the design of protocol for the synthesis of nanomaterials of different physico chemical features[6]. Currently, biological techniques for the synthesis of nanoparticles are reported as safe and environmental benign, they are also cost-effective and ensure the complete withdrawal of toxic chemicals [7].

Magnesium oxide nanoparticles are highly ionic nanoparticulate metal oxides with extraordinarily high surface areas and exceptional crystal morphologies[8]. Interfresh MgO nanoparticles unique optical, electronic, magnetic, thermal, mechanical, and chemical properties led to find its applications in wide areaof research[9].

Typical synthesis of MgO nanoparticles reported on chemical precipitation [10] and thermal evaporation[11]. These synthesis methods are sophisticated, constrained by the expense of equipment, utilization of high temperature, pressure, for synthesis of metal oxides. It has also been reported thatMgO NPs synthesized by that conventstechnique often yield a relatively small surface area and due to that itshow low reaction activity [12].

Recently, green chemistry on nanoscience attracted appreciable spouting research in many distinctive fields ofnanomedicine, which is committedto the creation, advancement and utility of nanomaterials for life science. Theultimate aim of this new field is to design and develop multifunctional materials of a nanoscale range that are gearing the medical fields [13,14]by making improvements to existing materials [15,16,17]. The tiny particles with the atom by atom arrangement consisting large surface to volume ratio competent to invasive with functional groups and reacting led to accomplishment of unique applications[18].

On the basis in the present investigation an attempt was made to synthesis magnesium oxide nanoparticles using phytochemistry approach and their application on anthelminthic has been demonstrated. In India soil-transmitted helminths infected nearly 25% of total global cases with 220.6 million children in need of prevention therapeutics[19]. Soil-transmitted helminth infections continue to plague large parts of the world with India a significant contributor to the burden of disease[20]. Resistance of anthelminthic drugs aggravate the disease incidence serious attempts are being undertaken by the researchers to explore alternate therapeutics[21].

Experimental details:

The present investigation is addressed the synthesis of magnesium oxide nanoparticles. Green principle mediated synthesis of magnesium oxide nanoparticles using precursor Magnesium nitrate and *Manilkarazapota* leaf extract has been used as a reducing agent to synthesize magnesium oxide nanoparticles.*Manilkarazapota* L. belongs to the family Sapotaceae, Sapodilla is the common name of *Manilkarazapota* which is distributed all over the world. Ethnopharmacological properties of *Manilkarazapota* has been well documented. Its anti-diarrheal, diuretic, and laxative has been demonstrated [22].

Healthy leaves of *Manilkarazapota* were collected from Thiruvalluvar University botanical garden,Serkkadu, Vellore, Tamil Nadu. The leaves were washed with tap water, one or two times, again wash with distilled water, and shade dried under room temperature for 10 days. Dried leaves were powdered by using a mechanical grinder. 5 g of *Manilkarazapota*leaves fine powder in a 500ml beaker consist 200 ml of distilled water and boiled for one hour at 100^{o}C. Freshly prepared *Manilkarazapota* leaf extract was used for the synthesis of MgONPs. In the experiment, 5 ml of freshly prepared *Manilkarazapota* leaf extract and 20ml of distilled water were added to a 250ml beaker and 5 grams of Magnesium Nitrate was added to the solution and heated at 80^{o}C with continuous stirring for 4 hours.Biogenesis of Magnesium oxide nanoparticles has been monitored by the color change of the solution. The yellow solution changed into yellowish brown in4 hours of reaction.

Characterization of Magnesium Oxide Nanoparticles:

Aliquots of reaction solutions were monitored by UV-2300 TECH COMP double-beam spectrophotometer in defined compartments, each for comparison and test solution fitted with quartz cuvettes of 1 cm path length. Synthesized Magnesium oxide NPs were centrifuged at 8000 rpm for 10 min for FTIR measurements. To obtain the pellet, the deposited residue was dried and grinded with KBr and analyzed by Thermo Nicolet Quator in the diffuse reflectance mode operating at a resolution of 4 cm-1. X-ray diffraction patterns of greenly synthesized Magnesium oxide NPs were obtained using a Siefert X-ray diffractometer operating at a voltage of 40 kV and a tube current of 30 mA with Cu-Kα radiation. Newly synthesized Magnesium oxide nanoparticles were prepared for TEM analysis by putting a drop of nanoparticle solution on carbon-coated copper grids and allowing water to evaporate. TEM observations were conducted on JEOL 3010 running on a 120Kv acceleration voltage. Scanning Electron Microscopy was subjected to analysis of the structural characteristics of newly formed Magnesium oxide nanoparticles. To confirm the percentage of the elemental composition of Magnesium oxide nanoparticles, EDAX analysis was undertaken with the same instrument.

Anthelminthic activity;

The Indian earthworms, *Pheretimaposthums* were collected from moist soil of the botanical garden in Thiruvalluvar University, Serkkadu, Vellore. The earthworms werebroughtto the laboratory within the half-hour along with moist soil. The earthworms were washed with saline water for removing the fecal matter. In-vitrostudy of anthelminthic activity was made using the suitable adult *Pheretimaposthumus* earthworms based on its physiological and anatomical resemblance with intestinal roundworms parasites of human beings. The investigation was carried out by revealing the worms to various dosages of magnesium oxide nanoparticles. The in-vitro studies were made according to the established protocol [23,24] 20ml of the solution containing four different dosages, each of aqueous *Manilkarazapota* leaf extract and Magnesium oxide nanoparticles (10,20,30, and 50mg/ml in distilled water) were prepared and ten worms were placed in it. Time for paralysis was recorded when no motility of any sort was observed except when the worms were shaken vigorously. The time for the death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water and thus worm lost their motility followed by fading away of their body color. The mean paralysis time and mean lethal time of each concentration were recorded.

Results and Discussion

Currently, metal oxide nanoparticles gaining momentous due to its physicochemical features which find application in diverse areas like catalysts [25] sensors [26] and medical application. Generation of nanostructured metal and metal oxide by biological resources have been intensively investigated to replace the conventional techniques[27- 31]. Magnesium oxide is an environmental benign, cost-effective and industrial concerned nanoparticle due its uniquefeaturesparticularly its unique refractive index,[32,33]. The unique surface chemical activity of magnesium oxide nanoparticles and their environmental friendliness have attracted researchers and explored its application as a catalyst, activities of anticancer and antimicrobial[34-37].

Present report demonstrates sapodilla leaf extract synthesized magnesium oxide nanoparticles in four hours reaction. The change in the intensity colorof the leaf extract was recorded as initial point of the formation of the MgONPs. The newly synthesized Magnesium oxide nanoparticles in aqueous suspension was analysed using UV-vis spectra in the range of 200-800nm (Fig. 1). The sample disclosed surface plasma resonance at 300 nm. The observed increasing shift of the UV–spectra of MgONPs may be due to the aggregation of the nano assemblage magnesium oxide. Appearance of denotes single peak that the prepared nanoparticlesis iso-morphological[38]. Thecolor change is due to the phytochemical-reduction by the leaf extract of *Manilkarazapota*with of the magnesium nitrate and it is clean for the biological synthesis of magnesium oxide nanoparticles.

The functional groups of the various metabolites present in the Manilkarazapota leaf extract are responsible for the stabilizing, bioreduction, and capping of newly synthesized magnesium oxide nanoparticles were identified between 4000 and 500 $cm^{-1.}$ Figure 2 shows the comparative FTIR spectra of synthesized magnesium oxide nanoparticles from the aqueous Manilkarazapota leaves extract. The spectra band show 3431 cm^{-1}, 2921 cm^{-1}, 2842 cm^{-1}, 1598 cm^{-1}, 1431 cm^{-1}, 1374 cm^{-1}, 1061 $cm^{-1,}$ 809 $cm^{-1,}$ 752 cm^{-1},601 cm^{-1} and the peak were shifted to 3422 cm^{-1}, 2850 cm^{-1},1646 cm^{-1},1381 cm^{-1},1110 cm^{-1},616 cm^{-1} respectively. The broad band observed at 3422 cm^{-1} was assigned to O-H stretching vibration hydroxyls overlapped with N-H stretching vibration of amines found in the polysaccharide. The small double peaks at 2922 cm^{-1} and 2850 cm^{-1} are considered for C-H stretching vibration in the alkene group in the phytochemicals. The median peak observed at 1641 cm^{-1} signified to the N-H bending mode of primary amine. The peak at 1381cm^{-1}corresponds to the C-H bending of the aldehyde group.The vital peak at 1110 cm^{-1}streching of C-O bond resulting carboxylic acid. The absorption peaks in the region between 487-677$cm^{-1}$are attributed to Mg-O compounds and the spectrum and the absorption bond 616 cm^{-1}.

High-Resolution Transmission Electron Microscopy (HR-TEM) has contributed to a further understanding of the structure and size of the newly formed magnesium oxide nanoparticles. A representative HR-TEM image of magnesium oxide nanoparticles is shown (Fig.5). The Magnesium Oxide Nanoparticles are rod and spherical shape, suggesting that the biomolecules of plant extract was capping. The SEM image of the *Manilkarazapota*synthesized magnesium oxide nanoparticles (Fig.4) showed that the magnesium oxide nanoparticles were spherical and the aggregation may be due to the interactions and Vander Waals forces between the magnesium oxide nanoparticles[39]. It is clean that the surface morphology of Magnesium oxide nanoparticles which are well dispersed. The EADX record shows the predominant peaks were in magnesium (Mg), oxygen (o), and nitrogen(N), and the signal for oxygen confirms the fact that Magnesium oxide Nanoparticles have been synthesized (Fig.6).

Magnesium oxide nanoparticles has been synthesized utilizing neem leaves [40] citrus lemon extract[41], *Punicagranatum* peels [42] Parthenium[43]*Brassica oleracea* and their utilizations on agriculture have been reported[44,45]. Abundance of phytochemical resources of plants considered potential to generate biogenic nanomaterials. The reductant and stabilizing molecules of plants provides fascinating applications in medical field as antimicrobial[46], diagnostics [47], biological labelling [48]. Green chemistry approach not only provides application on medical field it facilitates to overcome the limitations with respect to microbial synthesis. Microbial synthesis found to be time consuming tredious process of cell culture, extraction of biomolecules etc[49,50]. Plant biodiversity provides phytochemicals like flavones, aldehydes, amides, terpenoids, ketone are reported to have the property of reduction and stabilization of nanomaterials present in the report depicts alkanesandaromatic amines of the sapodilla leaf extract are responsible for the formation and stabilization magnesium oxide nanoparticles newly formed.

The particle size was also ascertained using particle size analyser and the results are presented (fig .7) and the particles in the size range of 30 to 90nm. The average particle size was 89.8nm.

Anthelmintic Activity Analysis:

Results of the anthelmintic activity depict that the biogenic magnesium oxide nanoparticles were found that the time taken for paralysis of *Pheretimaposthumus* was 6 min – 21 min and time taken for death was 5min – 70 min for different concentrations (10, 20,30, and 50 mg) of nano magnesium oxide particles. It was recorded that the Magnesium oxide nanoparticles demonstrates significant vermifugalactivity and the paralysis as well as the death of the worms in a time comparable to standard drugs used. Worm without treatment were alive throughout the experimental condition. The highest dosage of magnesium oxide nanoparticle solution (50 mg/ml) produced a paralytic effect much earlier and the time to death was shorter. Based on the above results, the activity of greenly synthesized magnesium oxide nanoparticles utilizing the leaf extracts of *Manilkarazapota* is greater when compared to the commercially available standard drug. The extracts of *Manilkarazapota* and magnesium oxide nanoparticles produced from it showed significant anthelmintic potential in a dose-dependent mannertable (1).

Histopathological studies

Histopathological studies were made using the experimental worms. The worms were cut in to pieces of 1 cm (fig. 8) which were place in neutral buffered formaldehyde. The tissues were subjected for process using automatic tissues processor sections were stained(fig.9) were stained by using an auto slide stainer HMS-70mm to examine the histopathological changes.Histopathological examination of the body of earthworms exposed to 5 µg/ cm2) revealed a loss of architecture. Neighboring cells in circular and longitudinal muscles are found to be discontinuousand it may be due to necrosis depending upon the effect of themagnesium oxide nanoparticles.

Gastrointestinal parasitic are pathogens of worldwide significance. Globally two billion people in developing countries, are estimated to be infected with soil-transmitted helminths, whilst helminth transmitted diseases are also a serious problem in livestock production globally causing significant economic losses and threatening food security[51,52,53]. These helminths cause malnutrition, malabsorption, iron-deficiency anemia and obstruction of the small intestine which leads to impairment in physical growth and cognitive and intellectual potential of children[54,55]. Besides this, helminthiasis is a common animal disease in developing countries, leading to a reduction in the production of milk[56].

Currently limited number of synthetic anthelmintic therapeutic are use in practice due to the threat of resistance development among parasites [57,58].the cost of the drugs for farmers in developing countries and lack of efficacy of existing drugs [59]. Hence its highly imperative to develop novel drugs which are urgently need. It has been observed that the survival of the parasite is potently inhibited by the newly synthesized magnesium oxide nanoparticles. Indeed, it has been observed that at 50mg/ml concentration completely a nested the adult survival.

Current investigation disclose that the stabilizing molecule of the newly formed magnesium oxide nanoparticles is derived the leaf extract of sapota,*Manilkarazapota*.Medicinal properties of sapota are due to chemical composition such as polyphenols, ascorbic acid, glycoside sapotinine etc. Further, it has reported that it is an excellent nutrient useful in the diseases like inflammation, pain, diarrhoea etc.

Albendazole is known to cause paralysis of worms so that they are expelled intothe faces of men and animals. Experimental biogenic magnesium oxide nanoparticles not only demonstrated this activity but also caused the early death of worms at all dosages compared to the drug. It is evident that magnesium oxide nanoparticles has depicts promising in vitro anthelmintic activity. Parasitic helminths affect animals and man, causing great hardship and stunted growth. Most diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites.The newly synthesized magnesium oxide nanoparticles may interfere with the parasites carbohydrate metabolism, inhibit their respiratory enzymes, block their neuromuscular action.

Conclusion

Environmental friendly, cost effective principle mediated synthesis of MgO nanoparticles has been accomplished. Biogenic MgO nanoparticles have been found to have potential anthelmintic activity with least concentration. Interestingly it has been achieved neither by dopping not by capping of any other molecules.

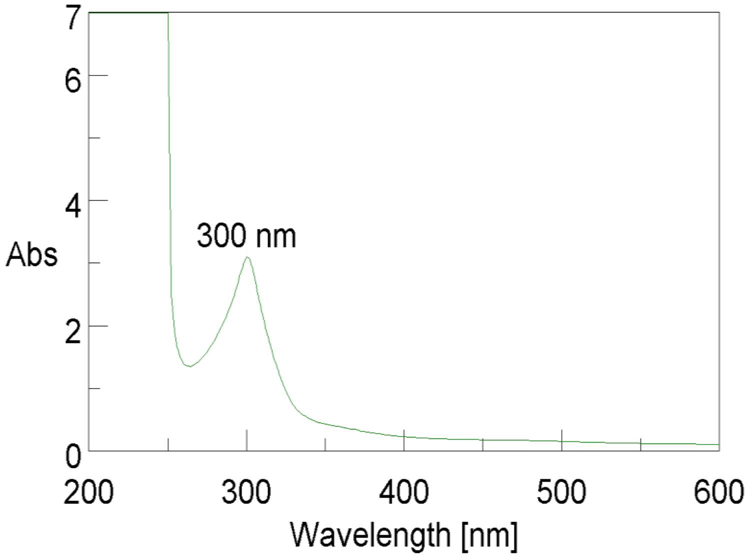


Figure no 1- UV- Vis spectrum shows Magnesium oxide nanoparticles' formation

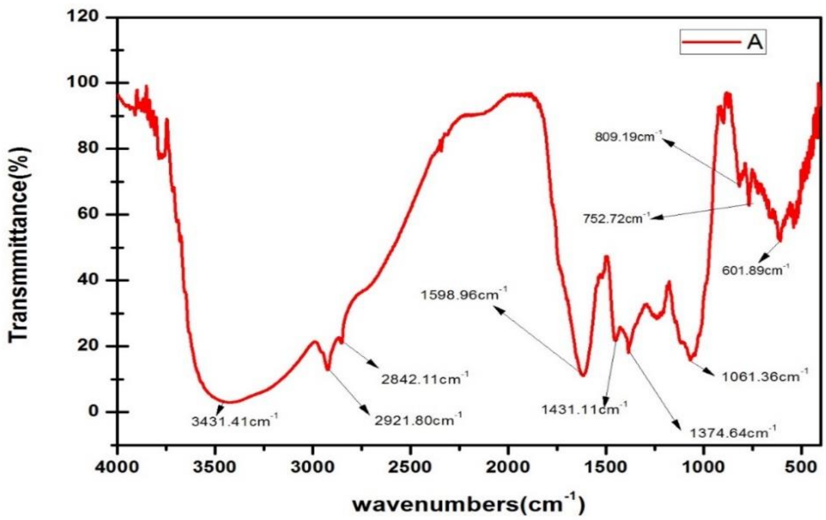
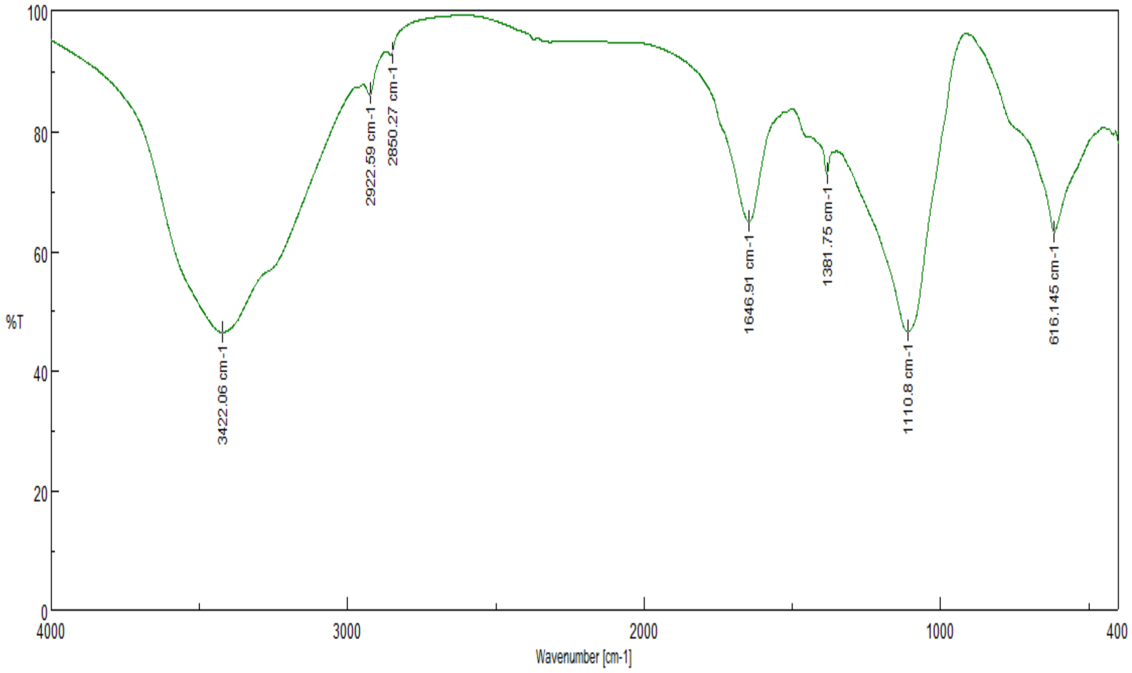
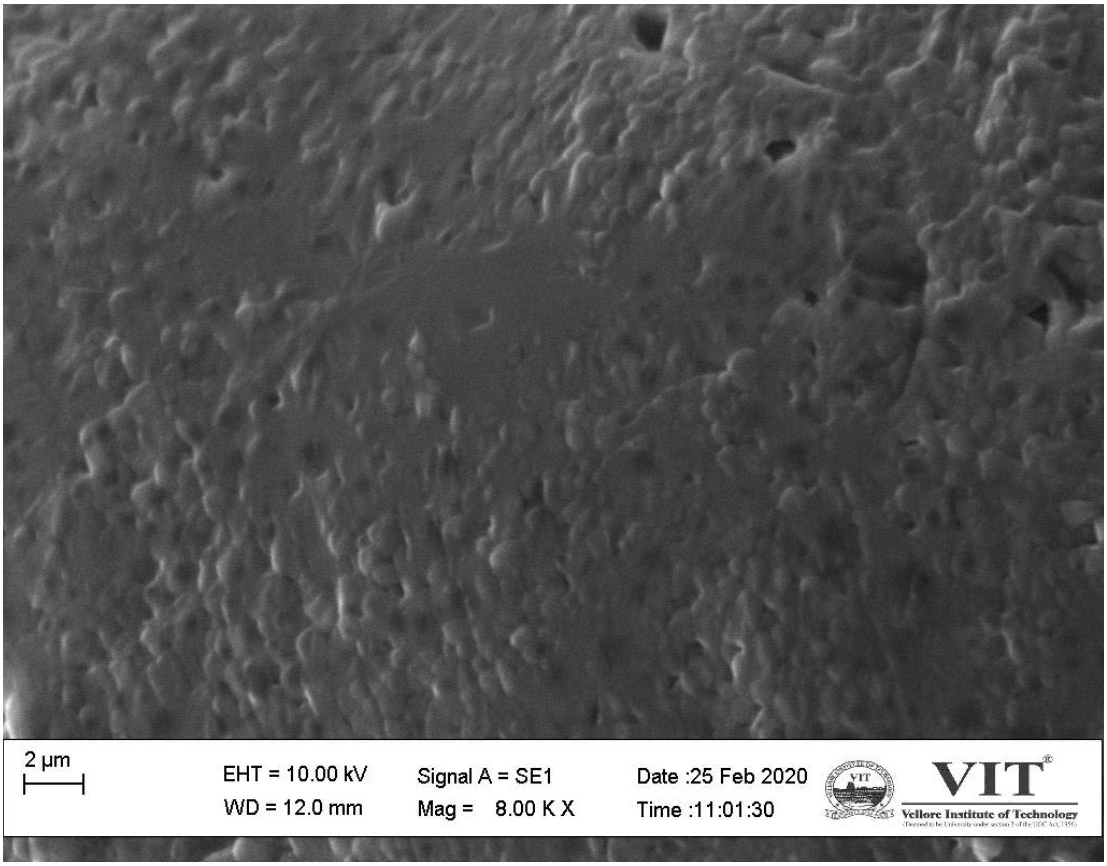


Figure 2 shows FTIR *Manilkarazapota* plant leaves extract



The figure no 3 shows FTIR newly synthesized Magnesium oxide nanoparticles



Figureno 4 shows Scanning Electron Microscope for Magnesium oxide Nanoparticles;

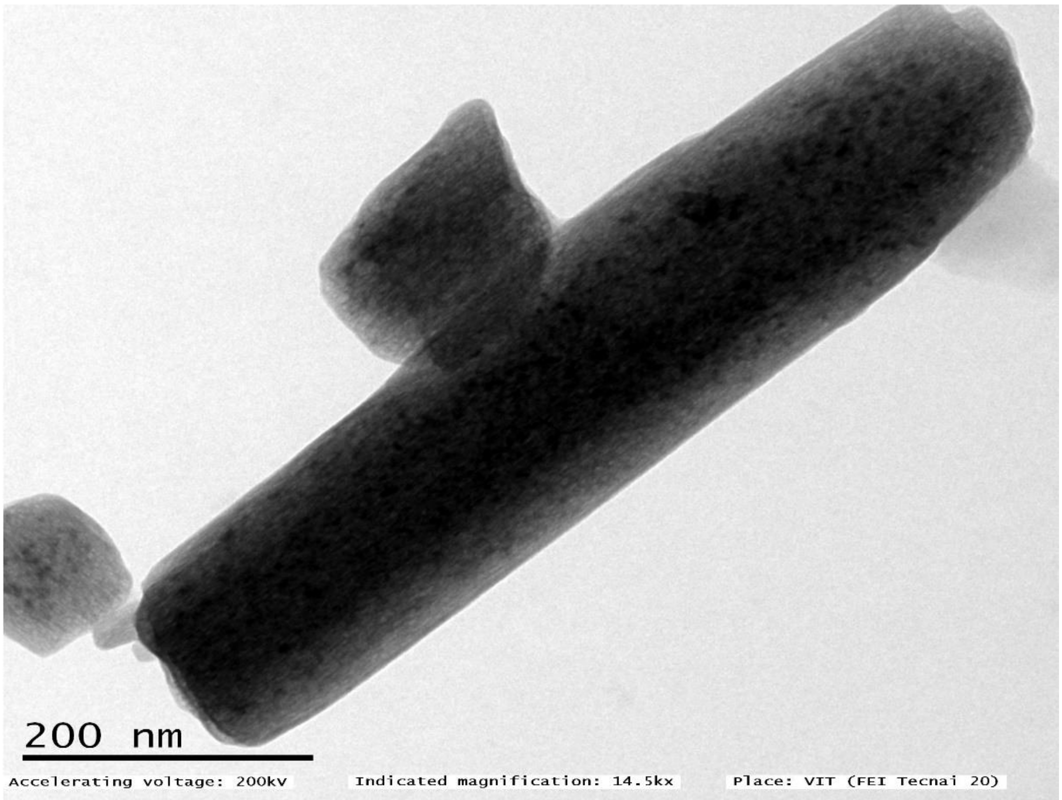


Figure no 5 shows Transmission Electron Microscope for Magnesium oxide nanoparticles.

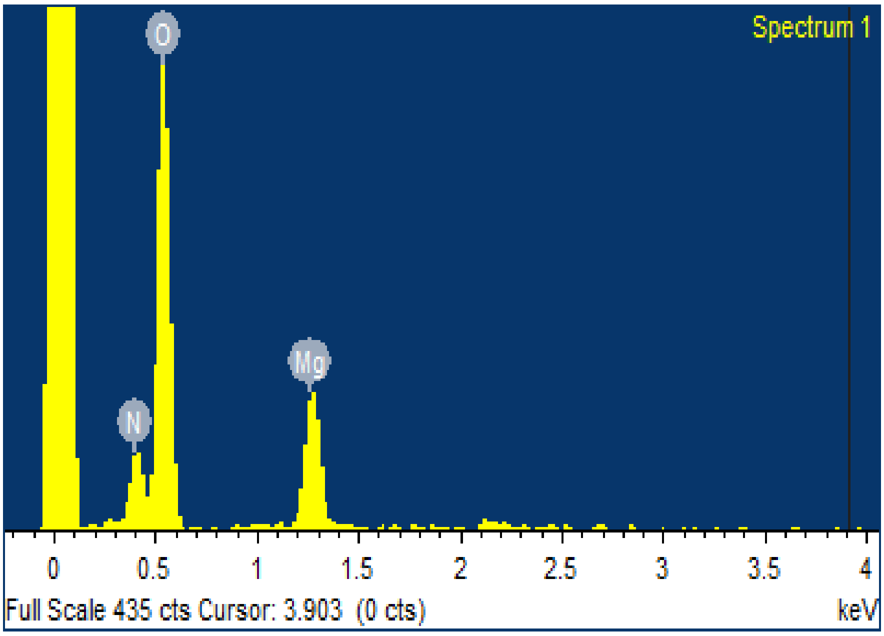


Figure no 6 shows EDAX for Magnesium oxide nanoparticles

Figure no 7 shows particle size analyzer of Magnesium oxide nanoparticles:

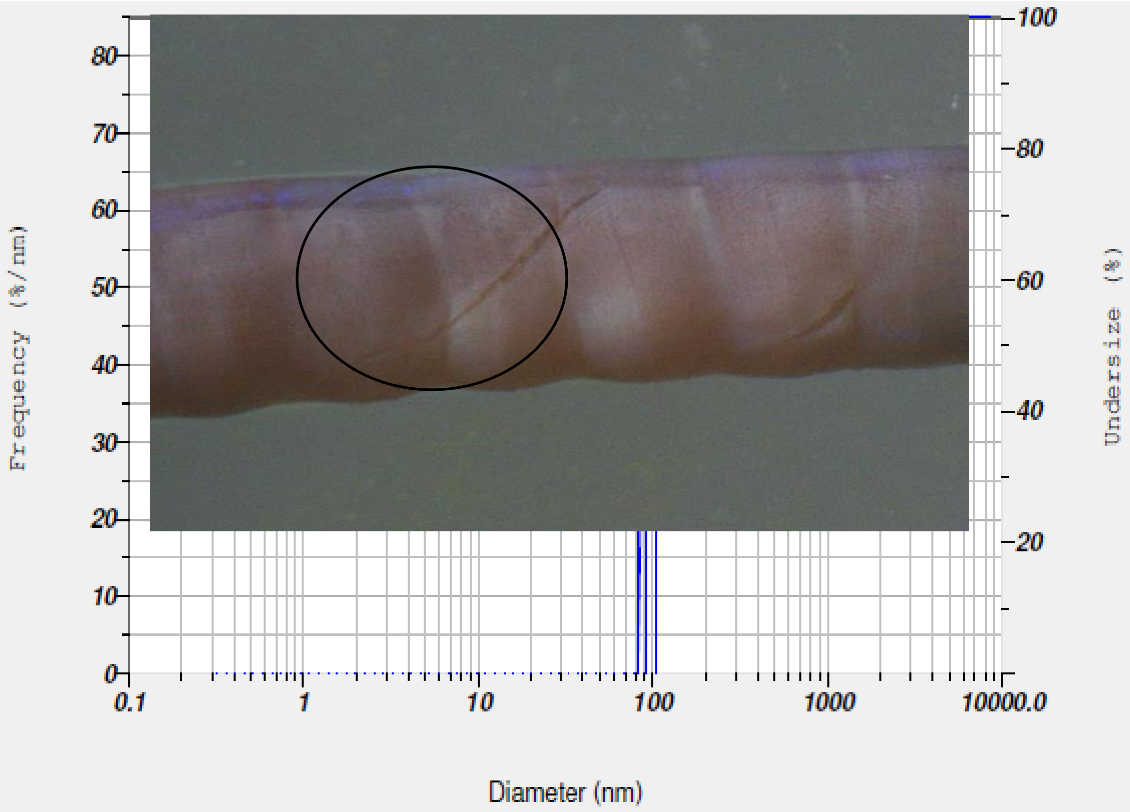
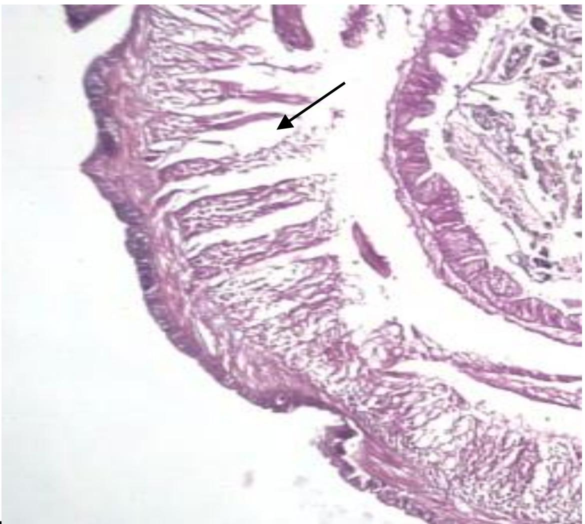


Figure no 8 shows surface lesions and peeling of the skin due to MgONPs exposure.



Figureno 9showsa Histopathological analysis of MgONPs treated subject showing discontinuous longitudinal muscles and necrosis.

Drug used	**Concentration mg/ml**	***Pheretima posthumous***		
		Paralysis Time(min)	Death (min)	Time

Standard	20		47.4±1.01	250.4±1.01
***Manilkarazapota* leaves extract**	10		63.8±1.16	296.2±1.6
	20		52.4±1.01	282.8±2.31
	30		46±1.41	255.2±1.72
	50		40.6±1.01	233.4±1.01
Magnesium oxide nanoparticles		10	21.4±1.2	124.2±0.74
		20	19.6±1.35	88.6±1.49
		30	13.2±1.32	57.8±1.16
		50	10.4±1.01	48.2±1.3

Table 1 shows the comparative anthelmintic potential of *Manilkarazapota*

Leaves extract and newly synthesized magnesium oxide nanoparticles in *Pheretima posthumous*

Reference

1. Ghormade, V., Deshpande, M. V., and Paknikar, K. M. (2011). Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechnol. Adv. 29, 792–803. doi: 10.1016/j.biotechadv.2011.06.007
2. Alaa Y G, Tawfiq M A, Akl M M and Muhand W A (2017) ARPN J. Agric. Biol. Sci. 10 293
3. Al-Gaashani R, Radiman S, Al-Douri Y, Tabet N and Daud AR (2012) J. Alloys Compd. 52 71
4. Krishnamoorthy, K., Moon, J. Y., Hyun, H. B., Cho, S. K., and Kim, S.-J. (2012). Mechanistic investigation on the toxicity of MgO nanoparticles toward cancer cells. J. Mater. Chem. 22, 24610–24617. doi: 10.1039/c2jm35087d
5. Martinez-Boubeta, C., Balcells, L., Cristofol, R., Sanfeliu, C., Rodriguez, E., Weissleder, R., (2010). Self-assembled multifunctional Fe/MgOnanospheres for magnetic resonance imaging and hyperthermia.Nanomed.Nanotechnol. Biol. Med. 6, 362–370. doi: 10.1016/j.nano.2009. 09.003
6. Rai M, Yadav A (2013) Plants as potential synthesizer of precious metal nanoparticles: progress and prospects. IET Nanobiotechnol 3:117–124
7. Okitsu K, Mizukoshi Y, Yamamoto TA, Maeda Y, Nagata Y (2007) Sonochemical synthesis of gold nanoparticles on chitosa. Lett Mater 61(16):3429–3431
8. Ravishankar RV, Jamuna BA (2011) Nanoparticles and their potential application as antimicrobials. Science against microbial pathogens.Communicating current research and technological advances. A. Me´ndez-Vilas (Ed.), pp 197–209
9. Ramanujam K, Sundrarajan M (2014) Antibacterial effects of biosynthesized MgO nanoparticles using ethanolic fruit extract of Emblicaofficinalis. J PhotochemPhotobiol, B 141:296–300
10. Panigrahi H, KunduS,Ghosh SK, Nath S, Pal T (2004) General method of synthesis for metal nanoparticles. J Nanopart Res 6:411– 414
11. Yang Q, Sha J, Wang L, Yang D (2006) MgO nanostructures synthesized by thermal evaporation. Mater SciEng C 26:1097–1101
12. Anthony KJP, Murugan M, Jeyaraj M, Rathinam NK, Sangiliyandi G (2014)

Synthesis of silver nanoparticles using pine mushroom extract: a potential antimicrobial agent against E. coli and B. subtilis. J IndEngChem 20:2325–2331

13. Salata OV (2004) Applications of nanoparticles in biology and medicine. J Nano Biotech 2:3–8. doi:10.1186/1477-3155-2-3
14. Nagarajan Sangeetha, KuppusamyKumaraguruArumugam (2013) Extracellular synthesis of Zinc Oxide nanoparticle using seaweeds of gulf of mannar. India J Nano Biotech 11:39. doi:10. 1186/1477-3155-11-39
15. MubayiAnamika, ChatterjiSanjukta, Prashant MR, WatalGeeta (2012) Evidence based green synthesis of nanoparticles. Adv Mat Lett 3(6):519–525. doi:10.5185/amlett.2012.Icn-ano.353
16. GeoffreyOzin A, CademartiriLudovico (2009) Nanochemistry, what is next? Small 5:1240–1244. doi:10.1002/smll.200900113
17. 17.P.ARevell, (2006) The biological effects of nanoparticles, Nanotechnology Perception Vol.2: 283–298.
18. .Alivisatos P, Barbara PF (1998) From molecules to materials: current trends and future directions. Adv Mater 10:1297–1336. doi:10. 1007/978-94-015-9576-6-4
19. Kumar Brajesh, SmitaKumari, Cumbal Luis, Debut Alexils, Camacho Javier, Hernandez-Gallegos Elisabeth, de Guadalupe Marı´a, Chavez-Lopez Marcelo Grijalva, Angulo Yolanda, Rosero Gustavo (2015) Pomosynthesis and biological activity of silver nanoparticles using Passifloratripartita fruit extracts. Adv Mater Lett 6:127–132. doi:10.5185/amlett.2015.5697
20. http://www.who.int/neglected_diseases/preventive_chemotherapy/sth/db/?units=minimal&region=all&country=all&countries=all&year=all Accessed on 31 Oct 2015
21. Lobo DA, Velayudhan R, Chatterjee P, Kohli H, Hotez PJ. The neglected tropical diseases of India and South Asia: review of their prevalence, distribution, and control or elimination. PLoSNegl Trop Dis. 2011;5:e1222.
22. Ayaz M, Junaid M, Subhan F, Ullah F, Sadiq A, Ahmad S, et al. Heavy metals analysis, phytochemical, phytotoxic and anthelmintic investigations of crude methanolic extract, subsequent fractions and crude saponins from Polygonumhydropiper L. BMC Complement Altern Med 2014; 14: 465
23. Antimicrobial activity of *Terminaliacatappa*, *Manilkarazapota*, *Piper beetle* leaf extract (2008), Indian J. Pharm.Sci., 70,390.
24. Wahab A Rahman, Rownea Lee, ShaidaFarizaSulaiman.(2011) In vitro anthelmintic activity of neem plant (Azadirachtaindica) extract against third stage Haemonchuscontortus larvae from goats. Global Veterinaria, 7(1): 22-26
25. Joshny J, Ramya Devi D, VedhaHari BN.(2012) Phytochemical and In-Vitro Anthelmintic Activity of Hydro Alcoholic Extract of Bougainvillea Glabra. Int J Pharm PharmSci, 4(2): 115-117.
26. Jin, H.; Zhao, X.; Wu, Z.; Cao, C.; Guo, L.(2017) Supercritical water synthesis of nano-particle catalyst on

TiO2 and its application in supercritical water gasification of biomass. J. Exp. Nanosci.12, 72–82.
27. Zhu, L.; Zeng, W. (2017) Room-temperature gas sensing of ZnO-based gas sensor: A review. Sens. Actuators, A Phys. 267, 242–261.
28. 27.Marina, B., Marcelo, G., Mariana, R., Declan, M.D., Janainad, Silva, Crespo, (2020). Green synthesis of zinc oxide nanoparticles: a review of the synthesis methodology and mechanism of formation. Sustain. Chem. Pharm. 15 https://doi.org/10.1016/j. scp.2020.100223, 100223.
29. 28.Anu, R., Krishna, Y., Sheeja, J., (2020). A comprehensive review on green synthesis of nature-inspired metal nanoparticles: mechanism, application and toxicity. J. Clean. Prod. https://doi.org/10.1016/j.jclepro.2020.122880, 122880.
30. 29.Sunday, A.A., Aderonke, S.F., Femi, A.F., Abel, K.O., 2020. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation.Heliyon 6 (7).https://doi.org/10.1016/j.heliyon.2020.e04508 e04508.
31. 30.Harish, C., Pragati, K., Elza, B., Saurabh, Y., 2020. Medicinal plants: treasure trove for green synthesis of metallic nanoparticles and their biomedical applications. Biocatalagricbiotechnol 24. https://doi.org/10.1016/j.bcab.2020.101518, 101518.
32. 31.Happy, A., Venkat, K.S., (2020). A review on anti-inflammatory activity of green synthesized zinc oxide nanoparticle: mechanism-based approach. Bioorg.Chem. 94 https://doi.org/10.1016/j.bioorg.2019.103423, 103423.
33. 32.Shahira, H.E.M., (2018). Bioprocessing strategies for cost effective large-scale biogenic synthesis of nano-MgO from endophytic Streptomyces coelicolor strain E72 as an anti-multi drug resistant pathogens agent. Sci. Rep. 8, 3820.https://www.nature.com/articles/s41598-018-22134-x.
34. 33.Muhammad, I.K., Muhammad, N.A., Naveed, A., Jawayria, N., Hira, M., Tahir, I.A., Muhammad, B.T., Mohammad, R.K., (2020). Green synthesis of magnesium oxide nanoparticles using Dalbergiasissoo extract for photocatalytic activity and antibacterial efficacy. Appl. Nanosci. https://doi.org/10.1007/s13204-020-01414-x.
35. 34.Abdallah Y, Ogunyemi SO, Abdelazez A,(2019) The green synthesis of MgOnano-flowers using Rosmarinusofficinalis L. (Rosemary) and the antibacterial activities against Xanthomonasoryzaepv. oryzae. BioMed Res Int.1–8.
36. Salehifar N, Zarghami Z, Ramezani M.(2016)A facile, novel and low temperature synthesis of MgOnanorods via thermal decomposition using new starting reagent and its photocatalytic activity evaluation. Mater Lett.167:226–229.
37. Suresh J, Pradheesh G, Alexramani V,(2018) Green synthesis and characterization of hexagonal shaped MgO nanoparticles using insulin plant (Costuspictus D. Don) leave extract and its antimicrobial as well as anticancer activity. Adv Powder Technol. 29: 1685–1694.

38. Zhang W, Tay HL, Lim SS,(2010) Supported cobalt oxide on MgO: highly efficient catalysts for degradation of organic dyes in dilute solutions. ApplCatal B Environ. 95:93–99.
39. Jeevanandam J, Chan YS, Danquah MK (2017) Biosynthesis and characterization of MgO nanoparticles from plant extracts via induced modified nucleation. New J Chem. https://doi.org/10.1039/C6NJ03176E
40. A. Sobczak-Kupiec, D. Malina, M. Zimowskaa, Z. Wzorek,(2011) Characterization of gold nanoparticles for various medical applications, Dig. J. Nanomater. Bios. 6 (2) 803–808.
41. Moorthy SK, Ashok CH, VenkateswaraRao K, Viswanathan C (2015) Synthesis and characterization of MgO nanoparticles by Neem leaves through green method. Mater Today Proc 2:4360–4368.
42. Awwad AM, Ahmad AL (2014) Biosynthesis, characterization, and optical properties of magnesium hydroxide and oxide nanoflakes using Citrus limon leaf extract. Arab J PhysChem 1(2):66
43. Sugirtha P, Divya R, Yedhukrishnan R, Suganthi KS, Anusha N, Ponnusami V, Rajan KS (2015) Green synthesis of magnesium oxide nanoparticles using Brassica oleracea and Punicagranatum peels and their anticancer and photocatalytic activity. Asian J Chem 27(7):2513–2517
44. Kumar D, Reddy Yadav LS, Lingaraju K, Manjunath K, Suresh D, Prasad D, Nagabhushana H, Sharma SC, Raja Naika H, Chikkahanumantharayappa NG (2015) Combustion synthesis of MgO nanoparticles using plant extract: structural characterization and photoluminescence studies. AIP Conf Proc1665:050145
45. Rani P, Kaur G, Rao KV (2020) Impact of green synthesized metal oxide nanoparticles on seed germination and seedling growth of Vigna radiate (Mung Bean) and Cajanuscajan (Red Gram). J InorgOrganometPolym. https://doi.org/10.1007/s10904-020-01551-4
46. Singh J, Kumar S, Alok A, Upadhyay SK, Rawat M, Tsang DCW, Bolan N, Kim K-H (2019) The potential of green synthesized zinc oxide nanoparticles as nutrient source for plant growth. J Clean Product 214:1061–1070.
47. 46.Malarkodi C, RajeshkumarS,Paulkumar K, Gnanajobitha G, Vanaja M, Annadurai Eco-friendly synthesis and characterization of gold nanoparticles using Klebsiellapneumoniae (2013) Journal of Nanostructure in Chemistry2013, 3:30 doi:10.1186/2193-8865-3-30.
48. 47.Rajeshkumar S, Malarkodi C, Paulkumar K, Vanaja M, Gnanajobitha G, Kannan C, Annadurai G Seaweed mediated synthesis of gold nanoparticles using *Turbinariaconoides* and its characterization (2013) Journal of Nanostructures in Chemistry 3 (44): 1-7.
49. 48.Yasmin A, Ramesh K,Rajeshkumar S Optimization and stabilization of gold nanoparticles by using herbal plant extract with microwave heating (2014) Nanoconvergence 1 (12) 1-7.

50. C Malarkodi, S Rajeshkumarand G Annadurai Detection of environmentally hazardous pesticide in fruit and vegetable samples using Gold nanoparticles Food control (2017).
51. M. Ponnanikajamideen, S. Rajeshkumar, M. Vanaja, G. AnnaduraiIn Vivo Type 2 Diabetes and Wound-Healing Effects of Antioxidant Gold Nanoparticles Synthesized Using the Insulin Plant *Chamaecostuscuspidatus* in Albino RatsCanadian Journal of Diabetes (2019), *43*(2), 82-89.
52. Adelere IA, Lateef A (2016) A novel approach to the green synthesis of metallic nanoparticles: the use of agro-wastes, enzymes, and pigments. Nanotechnol Rev 5:567–587
53. Keiser J, Utzinger J: (2008) Efficacy of current drugs against soil-transmitted helminth infections: Systematic review and meta-analysis. JAMA, 299(16):1937–1948.
54. Charlier J, van der Voort M, Kenyon F, Skuce P, Vercruysse J (2014) Chasing helminths and their economic impact on farmed ruminants. Trends Parasitol30(7):361–367.
55. Fitzpatrick JL: Global food security: (2013) The impact of veterinary parasites and parasitologists. Vet Parasitol 195(3–4):233–248
56. Blumenthal DS, Schultz MG: (1975) Incidence of intestinal obstruction in children infected with Ascarislumbricoides. Am J Trop Med Hyg 24(5):801.
57. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ:(2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006, 367(9521):1521–1532
58. Dhar D, Sharma R, BansalG:(1982) Gastro-intestinal nematodes in sheep in Kashmir. Vet Parasitol, 11(2):271–277
59. Sutherland IA, Leathwick DM: (2011) Anthelmintic resistance in nematode parasites of cattle: a global issue? Trends Parasitol, 27(4):176–181.
60. Sargison ND: (2012) Pharmaceutical treatments of gastrointestinal nematode infections of sheep—Future of anthelmintic drugs. Vet Parasitol, 189(1):79–84.