Anti- Diabetic and Cytotoxic Effect of Zinc Oxide Nanoparticles Synthesised Using *Boerhaavia diffusa*

Original Research Article

ABSTRACT

Introduction: Nanotechnology is the art of manipulating matter in terms of nano scale. It mainly aims at combining nanotechnology with drugs to improve the ability of a specific target. These materials are produced at nanoscale level as it is safe to administer in the body. *Boerhaavia diffusa* is a flowering plant which is also known as Punarnava. It is a herbal medicine that is used for pain relief. It has been used primarily for its anti-diabetic and diuretic properties. Zinc oxide nanoparticles is one of the prominent metal oxide nanoparticles which is used in applications of many research projects and industries. The synthesis using plant sources is used for the production of ZnO NPs due to its environmental, economic, and medicinal benefits.
Aim: The aim of this study is to determine the anti diabetic and cytotoxic effect of zinc oxide nanoparticles synthesised using *Boerhaavia diffusa*.
Materials and Methods: Zinc oxide nanoparticles were synthesised using *Boerhaavia diffusa* extract and Anti- diabetic activity was carried out using alpha amylase inhibitory activity and cytotoxic activity was done by brine shrimp lethality assay.
Results: The percentage of lethality was found to be 60%, 70%, 70%, 90%, 90%, and 0% in the 5 microliters, 10microliters, 20 microliters, 40 microliters, 80 microliters and 100 microliters concentration levels. Alpha amylase inhibitory activity was performed which showed better results indicating anti diabetic and cytotoxic activity. In anti diabetic inhibitory activity the values were 40%, 50%, 75%, 80% and 82% in 10mg/mL, 20mg/mL, 30mg/mL, 40mg/mL, 50mg/mL respectively. Both anti diabetic and cytotoxic effects showed prominent inhibitory effects.
Conclusion: Zinc oxide nanoparticles synthesised using *Boerhaavia diffusa* exhibited significant Anti -diabetic and cytotoxic activity in brine shrimp lethality assay showing emergence of a drug candidate for further research.

Keywords: Boerhaavia diffusa; Anti- diabetic activity; Cytotoxic activity; Zinc oxide nanoparticles.

1. INTRODUCTION:

Nanoscience and technology deals with the science and engineering of manipulation of particles at the level of single atoms and small groups of atoms. They have a wide range in research areas, which mainly involves structures, devices, and systems [1]. Nanotechnology has been used in the field of human health, which has been showing significant results, especially in the field of cancer treatment [2]. Nanomaterials are the important tools of nanotechnology [3]. The uniqueness of nanoparticles is mainly its size-dependent properties which makes it easier to administer in human beings [4].

Plant extracts that are biologically synthesised have a massive advantage over other biomolecules. Plant extracts have been used in the field of food, medicine, synthesis of nanoparticles and many more [5]. There are many techniques developed for the production of plant extracts based on the simplicity, cost,

and the type of extract content [4,6]. *Boerhaavia diffusa* is a plant that belongs to the family Nyctaginaceae. It is used as traditional medicine by indigenous people of many countries because of its protective role against inflammation, diabetes, cancer, gastrointestinal problems and many more [7]. The entire plant is believed to have numerous bioactive compounds which are responsible for the pharmacological activities [8]. *Boerhaavia diffusa* also possesses anti aging, disease prevention, and life strengthening properties [9].

Zinc oxide nanoparticles is considered as one of the most prominent metal oxide nanoparticles because of its wide range of applications [10]. It is used in applications of various research projects and industries [8]. The synthesis of plant sources is used for the production of ZnO NPs due to its environmental, economic, and medicinal benefits [8,11]. Zinc oxide nanoparticles are used in the production of disinfectant, anti-fungal, anticancer, antioxidant, anti-inflammatory and anti diabetics properties [12]. ZnO NPs are used in biomedicine, mainly in the fields that focus on anticancer and antibacterial [13]. Zinc is also known to keep the structural integrity of insulin [14]. Previously our team has published extensive research on various aspects [8,15–28]. This vast research experience has inspired us to do research on the anti diabetic and cytotoxic effect of zinc oxide nanoparticles synthesised using *Boerhaavia diffusa*.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

The fresh leaves of Boerhavia diffusa were collected in an unbiased manner and sampling was done by Randomised sampling method and washed thoroughly with distilled water. Only dried leaves of Boerhavia diffusa were included and other parts of the plant such as stem, root, flower were excluded in this study. About 1gm of clean dried leaves of Boerhavia diffusa was added to 50 mL of distilled water. The extract present in the conical flask is mixed well. This mixture is boiled at 60 degrees Celsius for 7 minutes with the help of a heating mantle. Then the boiled extract is filtered with the help of filter paper.

2.2 Synthesis of Zinc Oxide Nanoparticles

Boerhavia diffusa is treated with 0.507g of zinc sulphate and 90 mL of distilled water and it is placed in a semi-automatic shaker at 900 rpm. With the help of a double beam U-V spectrophotometer, the synthesis of nanoparticles for every one hour is noted. Then this formulation is placed in a centrifuge for 10 minutes. Now the synthesized nanoparticles which are settled at the bottom are collected. The randomized sampling method was done in an unbiased manner.

2.3 Anti- diabetic Activity

Antidiabetic activity Alpha mess in which the activity of behaviour decision using inculcate nanoparticles was done according to the standard method of (Ademilyi et al- 2013). In the artist you put the reaction mixture containing 500µl phosphate buffer (100mm, PH=6.8), 100µl alpha amylase(2µl) and varying concentration of extract (10 to 50mg/mL) was incubated at 37° for 20 minutes. The 200µl of 1% soluble starch(100 mm, phosphate buffer PH= 6.8) adds it as a substrate and incubates further at 37° for 30 minutes. 1000µl of 3,5 dinitro salicylic acid colour agent was added and boiled for 10 minutes. The absorbance of the resulting mixture was measured at 540 nanometre using a multiplate reader(multi thermo scientific, version 1.00.40). Acarbose at various concentrations(0.1-0.5 mg/mL) was used as standard. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity(%)= [1-AS/AC]× 100

where,

The absorbance is in the presence of a test substance and AC is the absorbance Control. The data were subjected to statistical analysis using one way analysis of variance and Duncan's.

2.4 Cytotoxic Activity

2g of iodine free salt was weighed and dissolved in 200mL of distilled water. 6 well plates were taken and 10-12 mL of saline water was filled. To that 10 nauplii were slowly added to each well(20µl, 40µl, 60µl, 80µl, 100µl). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24hours. After 24hours, the ELISA plates were observed and noted for the number of live

nauplii present and calculated by using the following formula.

3. RESULTS AND DISCUSSION

The results obtained from the alpha amylase inhibitory test, the % inhibition of zinc oxide nanoparticles was consistently better than the standard drug that is acarbose. The percentage inhibition gradually increased with the increase in concentration of the drug. It also increased in the same pattern of acarbose. Zinc oxide nanoparticles are relatively better than acarbose.

The results obtained from alpha glucosidase inhibitory activity, zinc oxide nanoparticles either showed the same % inhibition or slightly higher % inhibition compared to acarbose. At a concentration of 20µl and 30µl, The inhibitory action of zinc oxide nanoparticles was found to be slightly better than acarbose. Zinc oxide nanoparticles have maximum alpha amylase inhibition at 40µl and 50µl concentration. Its potential and drug action efficiency are almost similar to acarbose post which is the standard drug used as anti diabetic.

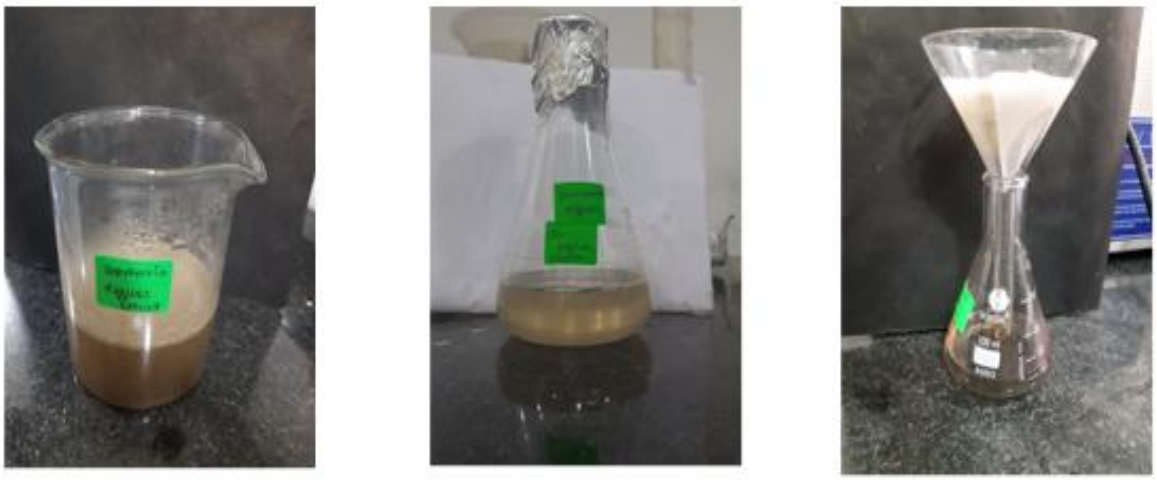

Fig. 1. Preparation of *Boerhaavia diffusa* extract mediated zinc oxide nanoparticles and the solution of *Boerhaavia diffusa* in zinc sulphate solution

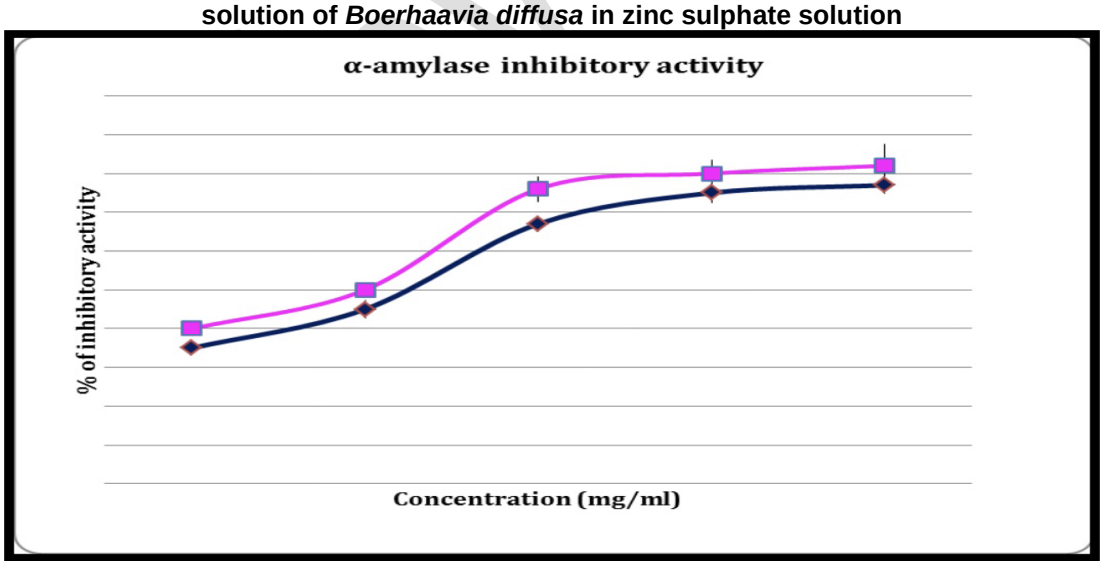

Fig. 2. The graph representing Anti diabetic activity of *Boerhaavia diffusa* mediated zinc oxide nanoparticles. data implies as mean±SEM with the level of statistical significance at p<0.05

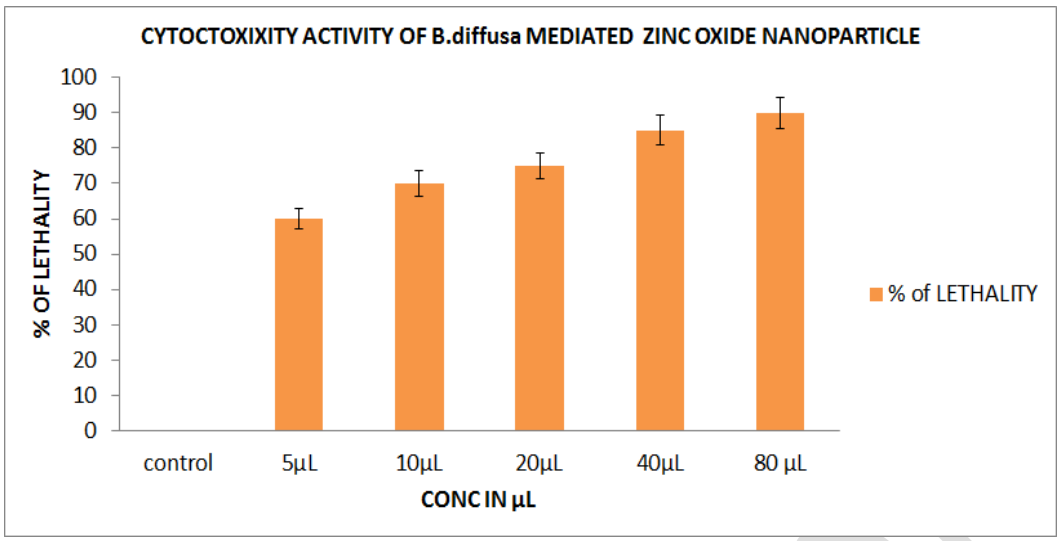

Fig. 3. The graph presenting cytotoxic activity of *Boerhaavia diffusa* mediated zinc oxide nanoparticles. X- axis represents concentrations and Y- axis represents lethality, data implies as mean±SEM

On the first day the nauplii were all alive. After 48 hours the number of nauplii changes were noted. The percentage of lethality was found to be 60%, 70%, 70%, 90%, 90%, and 0% in 5µl, 10µl, 20 µl 40µl, 80µl respectively. The maximum lethality was seen at 80 µl.

Zinc oxide nanoparticles are widely used for the production of disinfectants, anti fungal, anti cancer, anti oxidant, anti inflammatory and anti diabteic properties [29]. Previous studies show the use of plant mediated synthesis of nanoparticles which is considered as one of the most cost effective and environmental friendly methods to improve the antimicrobial activity of plant extracts [30]. It is seen that biosynthesis of ZnO nanoparticles was done with the help of extraction of Boerhavia diffusa leaves and the antimicrobial activity of synthesized nanoparticles was assessed [21,31]. It showed that ZnONPs could inhibit the activity of MRSA strains and could be aided as a potential antibacterial agent for cleaning and disinfection of MRSA in hospitals [23]. A study by Sadhan Kumar suggests that Green synthesis of zinc oxide nanoparticles was carried out using *Calotropis* leaf extract with zinc acetate salt in the presence of 2 M NaOH, which showed that [32]. The solution of zinc oxide nanoparticles is used as fertilizer because of its ability to grow faster. Nanofertilizers are used more commonly than conventional fertilizer because of its nutrient supplies and organic state [31]. A study shows that extracts of *Boerhaavia diffusa* leaves have been used to evaluate the antioxidant and hepatoprotective properties in the acetaminophen-induced liver damage model, which concludes that the leaf extracts of B.diffusa has exhibited hepatoprotective property against acetaminophen-induced liver damage that can be mediated through augmentation of antioxidant defenses [33]. Previous article shows that biological synthesis of zinc oxide nanoparticles from the petals extract of Rosa indica L was used to determine the efficacy against two dermatophytes which concluded that ZnO-NPs acts as a potential antifungal agent for treating skin infections as it reduces the inflammation [34]. An article shows that *Boerhaavia diffusa* has shown massive therapeutic activities such as example, diuresis, anticancer, anti-inflammation, hepatoprotection, and immunomodulation [35]. Although it is not that recognised in the market yet [9,34]. A study confirms that the entire plant of *Boerhaavia diffusa* contains numerous bioactive compounds which are responsible for many of its pharmacological activities [36]. A study by Pranati shows that plant extracts exhibited prominent immunomodulatory, immunosuppressive and anti-lymphoproliferative activities. Pharmacological studies on *Boerhaavia diffusa* confirms diuretic and anti-inflammatory [35,37] activities, which makes it more suitable in the treatment of inflammatory renal diseases [38]. Previously our team has published extensive research on various aspects [39 -52].

The limitations of the study is that *Boerhaavia diffusa* with zinc oxide nanoparticles is alone taken and silver, selenium, zinc and titanium are excluded. Other parts of *Boerhaavia diffusa* plant such as stem, flower and roots are not studied. Further studies should involve the use of *Boerhaavia diffusa* extract with many other nanoparticles and many other activities must be performed.

4. CONCLUSION

From the above study, it can be concluded that zinc oxide nanoparticles synthesised using *Boerhaavia diffusa* shows prominent anti diabetic and cytotoxic activity in brine shrimp lethality assay showing emergence of a drug candidate for further research [53-62]. It can also be used to perform antimicrobial, anti-inflammatory, antioxidant activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jaisankar AI, Arivarasu L. Free radical scavenging and anti-inflammatory activity of chlorogenic acid mediated silver nanoparticle [Internet]. Journal of Pharmaceutical Research International. 2020;106–12. Available:http://dx.doi.org/10.9734/jpri/2020/v32i1930715
2. Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine. Molecules [Internet]. 2019 Dec 27;25(1). Available:http://dx.doi.org/10.3390/molecules25010112
3. Priya SV, Vishnu Priya S, Shakeel Anjum M, Hariprasad G, Sravya T, Sai Pravalika T, et al. The role of job and personal resources in alleviating dentists' emotional dissonance in dental clinics of Hyderabad: a cross sectional study [Internet]. International Journal of Community Medicine And Public Health. 2020;7: 2672. Available:http://dx.doi.org/10.18203/2394-6040.ijcmph20202996
4. Salata OV. Applications of nanoparticles in biology and medicine. J Nanobiotechnology. 2004 Apr 30;2(1):3.
5. K S, Sarojini K, Arivarasu L, S SGA. Herbal formulation: Review of efficacy, safety, and regulations [Internet]. Vol. 11, International Journal of Research in Pharmaceutical Sciences. 2020.;11:1506–10. Available:http://dx.doi.org/10.26452/ijrps.v11ispl3.3467
6. Ocsoy I, Tasdemir D, Mazicioglu S, Tan W. Nanotechnology in Plants. Adv Biochem Eng Biotechnol. 2018;164:263–75.
7. Wu S, Rajeshkumar S, Madasamy M, Mahendran V. Green synthesis of copper nanoparticles using Cissus vitiginea and its antioxidant and antibacterial activity against urinary tract infection pathogens [Internet]. Artificial Cells, Nanomedicine, and Biotechnology. 2020;48:1153–8. Available:http://dx.doi.org/10.1080/21691401.2020.1817053
8. Kaur H. *Boerhaavia diffusa*: Bioactive compounds and pharmacological activities [Internet]. Biomedical and Pharmacology Journal. 2019;48:1675–82. Available:http://dx.doi.org/10.13005/bpj/1797
9. Mishra S, Aeri V, Gaur PK, Jachak SM. Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb:boerhavia diffusalinn [Internet]. BioMed Research International. 2014;2014:1–19. Available:http://dx.doi.org/10.1155/2014/808302
10. Sivaraj R, Pattanathu K S, Rajiv P, Narendhran S, Venckatesh R. Biosynthesis and characterization of Acalypha indica mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity [Internet]. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014;129:255–8. Available:http://dx.doi.org/10.1016/j.saa.2014.03.027
11. Faisal M, Saquib Q, Alatar AA, Al-Khedhairy AA. Phytotoxicity of nanoparticles. Springer; 2018;407.
12. M G, Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, et al. Phyllanthus reticulatus mediated

synthesis and characterization of silver nanoparticles and its antibacterial activity against gram positive and gram negative pathogens [Internet]. International Journal of Research in Pharmaceutical Sciences. 2019;10:3099–106. Available:http://dx.doi.org/10.26452/ijrps.v10i4.1603

13. Zhang Y, Nayak T, Hong H, Cai W. Biomedical applications of zinc oxide nanomaterials [Internet]. Current Molecular Medicine. 2013;13:1633–45. Available:http://dx.doi.org/10.2174/1566524013666131111130058
14. Jiang J, Pi J, Cai J. The advancing of zinc oxide nanoparticles for biomedical applications [Internet]. Bioinorganic Chemistry and Applications. 2018;2018:1–18. Available:http://dx.doi.org/10.1155/2018/1062562
15. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol. 2018; Oct;117:91–5.
16. Kanchi S, Ahmed S. Green Metal Nanoparticles: Synthesis, Characterization and their Applications. John Wiley & Sons; 2018;720.
17. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.–mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells [Internet]. Environmental Science and Pollution Research. 2020;27: 8166–75. Available:http://dx.doi.org/10.1007/s11356-019-07511-x
18. Perkampus H-H. UV-VIS Spectroscopy and Its Applications. Springer Science & Business Media. 2013;244.
19. Rajasekaran S, Damodharan D, Gopal K, Rajesh Kumar B, De Poures MV. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends [Internet]. Fuel. 2020;277:118166. Available:http://dx.doi.org/10.1016/j.fuel.2020.118166
20. Saravanan M, Arokiyaraj S, Lakshmi T, Pugazhendhi A. Synthesis of silver nanoparticles from Phenerochaete chrysosporium (MTCC-787) and their antibacterial activity against human pathogenic bacteria. Microb Pathog. 2018 Apr;117:68–72.
21. Karthik V, Arivarasu L, Rajeshkumar S. Hyaluronic acid mediated zinc nanoparticles against oral pathogens and its cytotoxic potential [Internet]. Journal of Pharmaceutical Research International. 2020;113–7. Available:http://dx.doi.org/10.9734/jpri/2020/v32i1930716
22. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. Arab J Gastroenterol. 2018 Jun;19(2):56–64.
23. Nimisha P, Assistant professor of Zoology, PSMO college, Tirurangadi, 676306. Evaluation of insecticidal activity of boerhavia diffusa against certain stored product insects [Internet]. International Journal of Advanced Research. 2019;7: 1252–5. Available:http://dx.doi.org/10.21474/ijar01/9618
24. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018 Oct;89(10):1241–8.
25. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. Arch Oral Biol. 2021 Feb;122:105030.
26. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry? Oral Surg Oral Med Oral Pathol Oral Radiol. 2021 Jul;132(1):118–9.
27. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Navarasampatti Sivaprakasam A. Compatibility of nonoriginal abutments with implants: evaluation of microgap at the implant–abutment interface, with original and nonoriginal abutments. Implant Dent. 2019 Jun;28(3):289.
28. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products [Internet]. Aquaculture. 2019;507:1–6. Available:http://dx.doi.org/10.1016/j.aquaculture.2019.04.004
29. Akash N, Arivarasu L, Rajeshkumar S. Anti-inflammatory and antioxidant potential

of hyaluronic acid mediated zinc nanoparticles [Internet]. Journal of Pharmaceutical Research International. 2020;33–7. Available:http://dx.doi.org/10.9734/jpri/2020/v32i2030727
30. Shankar SB, Barani Shankar S, Arivarasu L, Rajeshkumar S. Biosynthesis of hydroxy citric acid mediated zinc nanoparticles and its antioxidant and cytotoxic activity [Internet]. Journal of Pharmaceutical Research International. 2020;108–12. Available:http://dx.doi.org/10.9734/jpri/2020/v32i2630845
31. Chaudhuri SK, Malodia L. Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantea: characterization and its evaluation on tree seedling growth in nursery stage [Internet]. Applied Nanoscience. 2017;7:501–12. Available:http://dx.doi.org/10.1007/s13204-017-0586-7
32. Aathira CM, Arivarasu L, Rajeshkumar S. Antioxidant and anti-inflammatory potential of chromium picolinate mediated zinc oxide nanoparticle [Internet]. Journal of Pharmaceutical Research International. 2020;118–21. Available:http://dx.doi.org/10.9734/jpri/2020/v32i1930717
33. Olaleye MT, Tolulope Olaleye M, Akinmoladun AC, Ogunboye AA, Akindahunsi AA. Antioxidant activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa* Linn against acetaminophen-induced liver damage in rats [Internet]. Food and Chemical Toxicology. 2010;48:2200–5. Available:http://dx.doi.org/10.1016/j.fct.2010.05.047
34. Tiwari N, Pandit R, Gaikwad S, Gade A, Rai M. Biosynthesis of zinc oxide nanoparticles by petals extract of Rosa indica L., its formulation as nail paint and evaluation of antifungal activity against fungi causing onychomycosis [Internet]. IET Nanobiotechnology. 2017;11: 205–11. Available:http://dx.doi.org/10.1049/iet-nbt.2016.0003
35. Nasim I, Kamath K, Rajeshkumar S. Evaluation of the re-mineralization capacity of a gold nanoparticle-based dental varnish: An in vitro study [Internet]. Journal of Conservative Dentistry. 2020;23:390. Available:http://dx.doi.org/10.4103/jcd.jcd_315_20
36. Shree MK, Kavya Shree M, Arivarasu L, Rajeshkumar S. Cytotoxicity and antimicrobial activity of chromium picolinate mediated zinc oxide nanoparticle [Internet]. Journal of Pharmaceutical Research International. 2020;28–32. Available:http://dx.doi.org/10.9734/jpri/2020/v32i2030726
37. S K, Satheesha KS. In-Vitro antibacterial activity of black tea (*Camellia sinensis*) mediated zinc oxide nanoparticles against oral pathogens [Internet]. Bioscience Biotechnology Research Communications. 2020;13:2077–80. Available:http://dx.doi.org/10.21786/bbrc/13.4/66
38. Agrawal B, Das S, Pandey A. *Boerhaavia diffusa* Linn.: A review on its phytochemical and pharmacological Profile [Internet]. Asian Journal of Applied Sciences. 2011; 4:663–84. Available:http://dx.doi.org/10.3923/ajaps.2011.663.684
39. Pushpaanjali G, Geetha RV, Lakshmi T. Knowledge and awareness about antibiotic usage and emerging drug resistance bacteria among dental students. Journal of Pharmaceutical Research International. 2020 Aug 24;34–42.
40. Aathira CM, Geetha RV, Lakshmi T. Knowledge and awareness about the mode of transmission of vector borne diseases among general public. Journal of Pharmaceutical Research International. 2020 Aug 24;87–96.
41. Baskar K, Lakshmi T. Knowledge, attitude and practices regarding HPV vaccination among undergraduate and postgraduate dental students in Chennai. Journal of Pharmaceutical Research International. 2020 Aug 25;95–100.
42. Manya Suresh LT. Wound healing properties of aloe barbadensis miller-in vitro assay. Journal of Complementary Medicine Research. 2020;11(5):30–4.
43. First Report on Marine Actinobacterial Diversity around Madras Atomic Power Station (MAPS), India [Internet]. [cited 2021 Aug 31]. Available:http://alinteridergisi.com/article/first-report-on-marine-actinobacterial-diversity-around-madras-atomic-power-station-maps-india/
44. Physicochemical Profile of Acacia Catechu Bark Extract – An in Vitro Stud - International Journal of Pharmaceutical and Phytopharmacological Research [Internet]. [cited 2021 Aug 31]. Available:https://eijppr.com/article/physicoc

hemical-profile-of-acacia-catechu-bark-extract-an-in-vitro-stud
45. Lakshmi T. Antifungal activity of *Ficus racemosa* ethanolic extract against dermatophytes-an in vitro study. Journal of Research in Medical and Dental Science. 2021;9(2):191–3.
46. Awareness of Drug Abuse among Teenagers - International Journal of Pharmaceutical and Phytopharmacological Research [Internet]. [cited 2021 Aug 31]. Available:https://eijppr.com/article/awareness-of-drug-abuse-among-teenagers
47. Mangal CSK, Anitha R, Lakshmi T. Inhibition of Nitric oxide Production and Nitric oxide Synthase Gene Expression in LPS Activated RAW 264 .7 Macrophages by Thyme oleoresin from *Thymus vulgaris*. J Young Pharm. 2018;10(4):481.
48. COX2 Inhibitory Activity of Abutilon Indicum - Pharmaceutical Research and Allied Sciences [Internet]. [cited 2021 Aug 31]. Available:https://ijpras.com/article/cox2-inhibitory-activity-of-abutilon-indicum
49. Jibu RM, Geetha RV, Lakshmi T. Isolation, detection and molecular characterization of *Staphylococcus aureus* from Postoperative Infections. Journal of Pharmaceutical Research International. 2020 Aug 24;63–7.
50. Sindhu PK, Thangavelu L, Geetha RV, Rajeshkumar S, Raghunandhakumar S, Roy A. Anorectic drugs: an experimental and clinical perspective – A Review. Journal of Complementary Medicine Research. 2020;11(5):106–12.
51. Nivethitha R, Thangavelu L, Geetha RV, Anitha R, RajeshKumar S, Raghunandhakumar S. In Vitro Anticancer Effect of Sesamum Indicum Extract -. Journal of Complementary Medicine Research. 2020;11(5):99–105.
52. Mariona P, Roy A, Lakshmi T. Survey on lifestyle and food habits of patients with PCOS and obesity. Journal of Complementary Medicine Research. 2020;11(5):93–8.
53. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative evaluation of remineralizing potential of a paste containing bioactive glass and a topical cream containing casein phosphopeptide-amorphous calcium phosphate: An in vitro study. Pesqui Bras Odontopediatria Clin Integr. 2019 Mar 12;19(0):4668.
54. Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease. Clin Exp Pharmacol Physiol [Internet]. 2017 Mar [cited 2021 Sep 15];44(3). Available:https://pubmed.ncbi.nlm.nih.gov/28004401/
55. Malli SN, Selvarasu K, Jk V, Nandakumar M, Selvam D. Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases. Case Rep Dent [Internet]. 2019 Jan 22 [cited 2021 Sep 15];2019. Available:https://pubmed.ncbi.nlm.nih.gov/30805222/
56. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev [Internet]. 2014 Jun 30 [cited 2021 Sep 15];8(1). Available:https://pubmed.ncbi.nlm.nih.gov/25992232/
57. Menon S, Ks SD, RS, S R, Vk S. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces [Internet]. 2018 Oct 1 [cited 2021 Sep 15];170. Available:https://pubmed.ncbi.nlm.nih.gov/29936381/
58. Samuel SR, Acharya S, Rao JC. School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. J Public Health Dent [Internet]. 2020 Jan [cited 2021 Sep 15];80(1). Available:https://pubmed.ncbi.nlm.nih.gov/31710096/
59. Praveen K, Narayanan V, Muthusekhar MR, Baig MF. Hypotensive anaesthesia and blood loss in orthognathic surgery: a clinical study. Br J Oral Maxillofac Surg [Internet]. 2001 Apr [cited 2021 Sep 15];39(2). Available:https://pubmed.ncbi.nlm.nih.gov/11286449/
60. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. Int Endod J [Internet]. 2011 Jun [cited 2021 Sep 15];44(6). Available:https://pubmed.ncbi.nlm.nih.gov/21255047/
61. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact. 2019 Aug 1;308:206–15.
62. Kumar MS, Vamsi G, Sripriya R, Sehgal

PK. Expression of matrix metalloproteinases (MMP-8 and -9) in chronic periodontitis patients with and without diabetes mellitus. J Periodontol. 2006 Nov;77(11):1803–8.