

PTEROCARPUS SANTA MEDIATED SYNTHESIS OF SELENIUM NANOPARTICLES AND ITS CYTOTOXIC EFFECT.

RUNNING TITLE: Synthesis of selenium nanoparticles and its cytotoxic effect

ABSTRACT:

Introduction: Selenium is an important trace element in the human body that plays a role in nutrition and medicine. Selenium research is gaining traction, not only because of its technical applications, but also because of its novel position in life sciences. The brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts etc. *Pterocarpus santalinus*, with the common names red sanders, red saunders, red sandalwood, Rakt Chandan, and saunders wood, is a species of pterocarpus endemic to the southern Eastern ghats mountain range of south india. This tree is valued for the rich red colour of its wood. The aim of the present study is to find out the pterocarpus santa mediated synthesis of selenium nanoparticles and its cytotoxic effect.

Materials and methods: Selenium nanoparticles were synthesized using pterocarpus santa and its cytotoxic effect was done in brine shrimp and its lethality is checked.

Results: cytotoxic activity and the number of nauplii were all alive on the first day. After 24 hrs the number of nauplii were dead in all wells except in the 5 μ l well. The percentage of lethality increased from 50%,100%,100%,100%,100% in the 5 μ L to 80 μ L respectively.

Note // It is preferable to extract the value of LC50

Conclusion: pterocarpus Santa mediated synthesis of selenium nanoparticles showed better cytotoxic effect in brine shrimp lethality assay showing emergence of a drug candidate for future research.

Key words: Pterocarpus santa; brine shrimp; cytotoxic activity; selenium nanoparticles; lethality test

INTRODUCTION:

Nanotechnology, or the manufacture of structures and devices at the molecular stage, is a multidisciplinary research discipline that is rapidly developing.(1). There is an increasing interest in nanotechnology and its applications in a variety of fields, especially medicine for diagnostic, therapeutic, and research biomedical tools. It can be described as any method or technique for producing nanoscale materials with particle sizes ranging from 1 to 100 nanometers(2). Nanomaterials are now the most advanced, both in terms of scientific understanding and industrial applications. Nanoparticles were first studied a decade ago because of their size-dependent physical and chemical properties. They've now reached an era of commercial exploration (2,3).

Nanoparticles (NPs) are used to minimize toxicity, increase bioactivity, improve targeting, and monitor the release profile of the encapsulated moiety in a variety of ways. Inorganic NPs of metals such as Ag, Au, Ce, Fe, Se, Ti, and Zn hold a special position among NPs due to their unusual bioactivities in nanoforms. Selenium (Se) is an essential trace mineral.(4).Due to their low toxicity and high biocompatibility, selenium nanoparticles (SeNPs) have attracted attention and are widely accepted in biomedicine and food science(5). Several studies have shown that selenium nanoparticles have anticancer, antioxidant, antibacterial, and anti-biofilm properties in recent years. These nanoparticles have shown to have impressive antimicrobial activity against pathogenic bacteria, fungi, and yeast.(6).

Pterocarpus santalinus, also known as Red sanders, is a member of the Fabaceae family(7). The plant is predominantly used to treat skin disorders, oral diseases, cough, pyrexia, diarrhoea, dysentery, and hyper nervous activity, as well as acting as an anti-hemorrhagic, anti-inflammatory, anti-bacterial, anti-cancer, and hepatoprotective agent.(8). *Artemia* (brine shrimp) is zooplankton that is used to feed larval fishes (9). *Artemia* have one thing in common: they are extremely adaptable to hypersaline conditions like permanent salt lakes, coastal lagoons, and man-made salt pans. They play a crucial role in the food chain's energy flow in the marine environment. (10).

In the previous study (11) the anti oxidant, antimicrobial, anti diabetic, anti inflammatory and analgesic activities of extracts from stem wood of *pterocarpus marsupium roxburgh* was done. (12) had done a study on terpenoids of *pterocarpus santalinus* (isoptercarpolone, pterocarptriol and pterocardiolone)besides the known β -eudesmol, pterocarpol and cryptomeridiol. Their structures have been determined by spectral and chemical studies. The study conducted (13) used to check the cytotoxicity of selenium nanoparticles in rat dermal fibroblasts. Previous studies

also state the similar effects (14). Our team has extensive knowledge and research experience that has translated into high quality publications (15–19), (20), (21), (15), (22), (23), (24), (25) (17, 26, 27), (28–32).

The aim of the present study is to synthesize extract of selenium nanoparticles using pterocarpus Santa and see its cytotoxic effect.

MATERIALS AND METHODS:

Green synthesis of Nanoparticle

The extract preparation was done by taking 0.5g of red sandal selenium mixed with 50ml of distilled water. The extract was boiled for 10mins at 55° Celsius and it had been filtered. Again sodium selenite was mixed in 50ml of distilled water and mixed with filtered pterocarpus santa extract. The red sandal selenium extract was kept in the shaker for an hour and reading was taken for its cytotoxic effect. **Note (Mention the source that contains the method of work)**



Fig-1 : Synthesis of P.santa Mediated Selenium nanoparticle

BRINE SHRIMP LETHALITY ASSAY:

Salt water preparation :

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 (5 μ L, 10 μ L, 20 μ L, 40 μ L, 80 μ L). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the plates were observed and noted for number of live nauplii present and calculated by using following formula,

$$\text{Number of dead nauplii} / \text{number of dead nauplii} + \text{number of live nauplii} \times 100$$

Note (Mention the source that contains the method of work)

RESULTS:

On the first day of the cytotoxic action, all of the nauplii were alive. After 24 hrs the number of nauplii were dead in all wells except in the 5 μ l well. The percentage of lethality increased from 50%, 100%, 100%, 100%, 100% in the 5 μ L, 10 μ L, 20 μ L, 40 μ L, 80 μ L respectively (Figure 2).

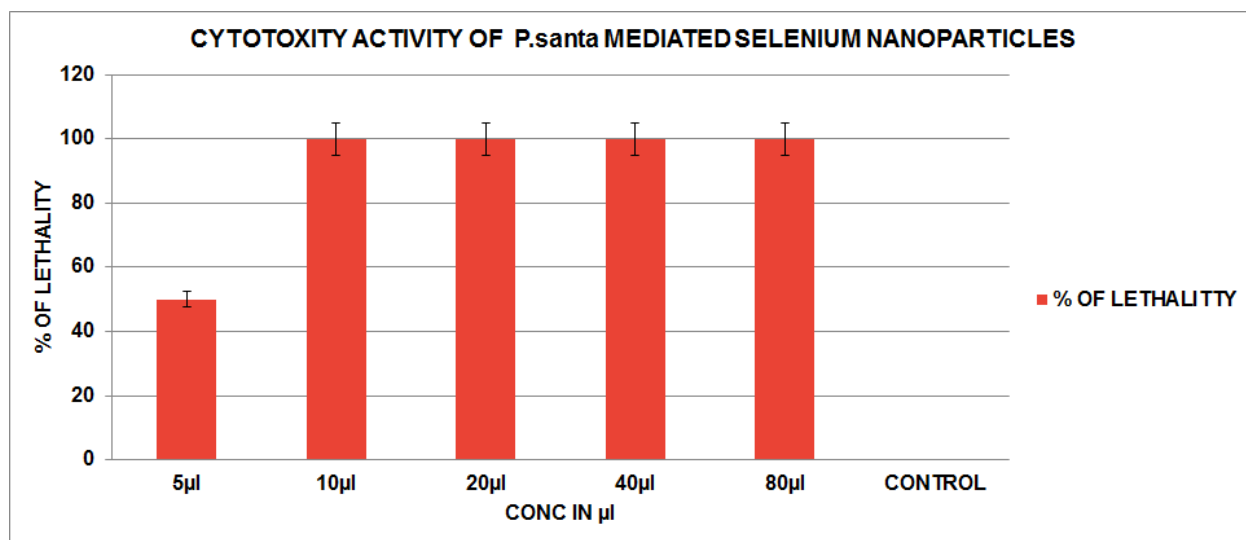


Figure-2 : The given figure represents the cytotoxic activity of brine shrimp using selenium nanoparticle extract where the X axis represents the concentration in μl and the Y axis represents the % of lethality of the brine shrimp nauplii, data implies as mean \pm SEM

NOTE // The results are few, please rewrite it again in a way that provides all the information about the search results

DISCUSSION:

Pterocarpus santa mediated synthesis of selenium nanoparticles extract has a positive outcome in this present study. It has shown that it has a potent effect on cytotoxic activity. Many studies have been conducted in this activity but with other nanoparticles like silver nanoparticles etc were used(33). The advantage of the selenium nanoparticle is that it is cost- efficient, high yield in reactions and less time consuming. Protein, peptides, and a number of other reducing agents are used to produce various forms of Se nanoparticles.(34). The study done by (35) evaluated the antioxidant and cytotoxic effect of selenium nanoparticles (Se NPs) biosynthesized by a newly isolated marine bacterial strain bacillus sp. MSh-1.

In contrast to other selenospecies, Se nanoparticles have been identified as novel compounds with excellent antioxidant properties and lower toxicity.(36). Because of their low toxicity and high stability, selenium nanoparticles (SeNPs) are widely accepted and recommended for use in a variety of scientific disciplines.(37). A pharmacological dose of SeNPs could lead to cytotoxicity and induce cell death through apoptosis and extrinsic pathways contributing to SeNP-induced apoptosis in BRL cells.(38) . In a rodent model, the use of SeNPs significantly decreases the death caused by acute Se toxicity by up to four times. Furthermore, as demonstrated by biomarkers of hepatotoxicity, the liver injuries associated with high doses of Se are significantly decreased when SeNPs are used.(4).

The brine shrimp lethality bioassay is a straightforward cytotoxicity test for bioactive chemicals with a high throughput. It is based on the ability of test compounds to destroy brine shrimp, a basic zoological organism (*Artemia salina*). (39).

Previously our institution has done several studies which include (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53)

The major limitation of the study is that it is conducted *in vitro*, so it cannot be assumed that the results of the cytotoxic activity could be translated into clinical effectiveness. In future studies, *in vivo* studies are recommended with people's recommendation values as well.

NOTE // The discussion is few. Please rewrite it again in a way that provides and covers all the information and reasons about the search results

CONCLUSION:

In the present study the brine shrimp nauplii showed that the pterocarpus santa mediated synthesis of the selenium nanoparticles showed better cytotoxic activity as the number of nauplii showed the percentage of the lethality by the increased concentration of the selenium nanoparticles extract (54-63). The Pterocarpus santa mediated synthesis of selenium nanoparticles showed potential cytotoxic effect in brine shrimp lethality assay showing emergence of a drug candidate for future research.

REFERENCES:

1. Emerich DF, Thanos CG. Nanotechnology and medicine. *Expert Opin Biol Ther.* 2003 Jul;3(4):655–63.
2. Ibrahim H. Nanotechnology and Its Applications to Medicine: an over view [Internet]. Vol. 113, *QJM: An International Journal of Medicine.* 2020. Available from: <http://dx.doi.org/10.1093/qjmed/hcaa060.008>
3. Salata OV. Applications of nanoparticles in biology and medicine. *J Nanobiotechnology.* 2004 Apr 30;2(1):1–6.
4. Khurana A, Tekula S, Saifi MA, Venkatesh P, Godugu C. Therapeutic applications of selenium nanoparticles. *Biomed Pharmacother.* 2019 Mar;111:802–12.
5. Gunti L, Dass RS, Kalagatur NK. Phytofabrication of Selenium Nanoparticles From *Emblca officinalis* Fruit Extract and Exploring Its Biopotential Applications: Antioxidant,

- Antimicrobial, and Biocompatibility [Internet]. Vol. 10, *Frontiers in Microbiology*. 2019. Available from: <http://dx.doi.org/10.3389/fmicb.2019.00931>
6. Vahdati M, Tohidi Moghadam T. Synthesis and Characterization of Selenium Nanoparticles-Lysozyme Nanohybrid System with Synergistic Antibacterial Properties. *Sci Rep*. 2020 Jan 16;10(1):510.
 7. Khare CP. *Pterocarpus santalinus* Linn. f [Internet]. *Indian Medicinal Plants*. 2007. p. 1–1. Available from: http://dx.doi.org/10.1007/978-0-387-70638-2_1298
 8. Navada KK, Vittal RR. Ethnomedicinal value of *Pterocarpus santalinus* (Linn. f.), a Fabaceae member [Internet]. Vol. 14, *Oriental Pharmacy and Experimental Medicine*. 2014. p. 313–7. Available from: <http://dx.doi.org/10.1007/s13596-014-0168-0>
 9. Perugini P, Simeoni S, Scalia S, Genta I, Modena T, Conti B, et al. Effect of nanoparticle encapsulation on the photostability of the sunscreen agent, 2-ethylhexyl-p-methoxycinnamate. *Int J Pharm*. 2002 Oct 10;246(1-2):37–45.
 10. Arulvasu C, Jennifer SM, Prabhu D, Chandhirasekar D. Toxicity effect of silver nanoparticles in brine shrimp *Artemia*. *ScientificWorldJournal*. 2014 Jan 2;2014:256919.
 11. Pant D, Pant N, Saru D, Yadav U, Khanal D. Phytochemical screening and study of anti-oxidant, anti-microbial, anti-diabetic, anti-inflammatory and analgesic activities of extracts from stem wood of *Pterocarpus marsupium* Roxburgh [Internet]. Vol. 6, *Journal of Intercultural Ethnopharmacology*. 2017. p. 1. Available from: <http://dx.doi.org/10.5455/jice.20170403094055>
 12. Kumar N, Ravindranath B, Seshadri TR. Terpenoids of *Pterocarpus santalinus* heartwood [Internet]. Vol. 13, *Phytochemistry*. 1974. p. 633–6. Available from: [http://dx.doi.org/10.1016/s0031-9422\(00\)91365-7](http://dx.doi.org/10.1016/s0031-9422(00)91365-7)
 13. Ramos JF, Webster TJ. Cytotoxicity of selenium nanoparticles in rat dermal fibroblasts. *Int J Nanomedicine*. 2012 Jul 23;7:3907–14.
 14. 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. p. 108–12. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i2630845>
 15. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. *Enzyme Microb Technol*. 2018 Oct;117:91–5.
 16. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. *Biocatal Agric Biotechnol*. 2019 May 1;19:101138.

17. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of *Enterococcus* sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. *Environ Sci Pollut Res*. 2020 Mar 1;27(8):8166–75.
18. Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using *Gymnema sylvestre* leaf extract and evaluation of its antibacterial activity [Internet]. Vol. 32, *South African Journal of Chemical Engineering*. 2020. p. 1–4. Available from: <http://dx.doi.org/10.1016/j.sajce.2019.11.005>
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. *Fuel*. 2020 Oct 1;277:118166.
20. Santhoshkumar J, Sowmya B, Venkat Kumar S, Rajeshkumar S. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of *Passiflora caerulea*. *S Afr J Chem Eng*. 2019 Jul;29:17–23.
21. Raj R K, D E, S R. β -Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line. *J Biomed Mater Res A*. 2020 Sep;108(9):1899–908.
22. 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.
23. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells. *Hum Exp Toxicol*. 2019 Jun 1;38(6):694–702.
24. Ezhilarasan D, Sokal E, Najimi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets. *Hepatobiliary Pancreat Dis Int*. 2018 Jun;17(3):192–7.
25. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. *Arab J Gastroenterol*. 2018 Jun;19(2):56–64.
26. Gomathi AC, Xavier Rajarathinam SR, Mohammed Sadiq A, Rajeshkumar S. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of *Tamarindus indica* on MCF-7 human breast cancer cell line. *J Drug Deliv Sci Technol*. 2020 Feb 1;55:101376.
27. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. *Drug Dev Res*. 2019 Sep;80(6):714–30.
28. 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.
29. 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.

30. 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.
31. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med. 2019 Feb;48(2):115–21.
32. 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):289–95.
33. Srinivasan R, Vigneshwari L, Rajavel T, Durgadevi R, Kannappan A, Balamurugan K, et al. Biogenic synthesis of silver nanoparticles using Piper betle aqueous extract and evaluation of its anti-quorum sensing and antibiofilm potential against uropathogens with cytotoxic effects: an in vitro and in vivo approach. Environ Sci Pollut Res Int. 2018 Apr;25(11):10538–54.
34. Husen A, Siddiqi KS. Plants and microbes assisted selenium nanoparticles: characterization and application. J Nanobiotechnology. 2014 Aug 16;12:28.
35. Forootanfar H, Adeli-Sardou M, Nikkhoo M, Mehrabani M, Amir-Heidari B, Shahverdi AR, et al. Antioxidant and cytotoxic effect of biologically synthesized selenium nanoparticles in comparison to selenium dioxide. J Trace Elem Med Biol. 2014 Jan;28(1):75–9.
36. Zhai X, Zhang C, Zhao G, Stoll S, Ren F, Leng X. Antioxidant capacities of the selenium nanoparticles stabilized by chitosan. J Nanobiotechnology. 2017 Jan 5;15(1):4.
37. Cittrarasu V, Kaliannan D, Dharman K, Maluventhen V, Easwaran M, Liu WC, et al. Green synthesis of selenium nanoparticles mediated from Ceropegia bulbosa Roxb extract and its cytotoxicity, antimicrobial, mosquitocidal and photocatalytic activities [Internet]. Vol. 11, Scientific Reports. 2021. Available from: <http://dx.doi.org/10.1038/s41598-020-80327-9>
38. Wang H, He Y, Liu L, Tao W, Wang G, Sun W, et al. Prooxidation and Cytotoxicity of Selenium Nanoparticles at Nonlethal Level in Sprague-Dawley Rats and Buffalo Rat Liver Cells. Oxid Med Cell Longev. 2020 Aug 14;2020:7680276.
39. Wu C. An important player in brine shrimp lethality bioassay: The solvent. J Adv Pharm Technol Res. 2014 Jan;5(1):57–8.
40. 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.

41. 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.
42. 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.
43. Manya Suresh LT. Wound Healing Properties of Aloe Barbadensis Miller-In Vitro Assay. *Journal of Complementary Medicine Research*. 2020;11(5):30–4.
44. First Report on Marine Actinobacterial Diversity around Madras Atomic Power Station (MAPS), India [Internet]. [cited 2021 Aug 31]. Available from: <http://alinteridergisi.com/article/first-report-on-marine-actinobacterial-diversity-around-madras-atomic-power-station-maps-india/>
45. Physicochemical Profile of Acacia Catechu Bark Extract – An in Vitro Stud - *International Journal of Pharmaceutical and Phytopharmacological Research* [Internet]. [cited 2021 Aug 31]. Available from: <https://eijppr.com/article/physicochemical-profile-of-acacia-catechu-bark-extract-an-in-vitro-stud>
46. 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.
47. Awareness of Drug Abuse among Teenagers - *International Journal of Pharmaceutical and Phytopharmacological Research* [Internet]. [cited 2021 Aug 31]. Available from: <https://eijppr.com/article/awareness-of-drug-abuse-among-teenagers>
48. 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.
49. COX2 Inhibitory Activity of Abutilon Indicum - *Pharmaceutical Research and Allied Sciences* [Internet]. [cited 2021 Aug 31]. Available from: <https://ijpras.com/article/cox2-inhibitory-activity-of-abutilon-indicum>
50. 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.
51. 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.
52. 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.

53. 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.
54. 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.
55. 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 from: <https://pubmed.ncbi.nlm.nih.gov/28004401/>
56. 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 from: <https://pubmed.ncbi.nlm.nih.gov/30805222/>
57. 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 from: <https://pubmed.ncbi.nlm.nih.gov/25992232/>
58. Menon S, Ks SD, R S, 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 from: <https://pubmed.ncbi.nlm.nih.gov/29936381/>
59. 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 from: <https://pubmed.ncbi.nlm.nih.gov/31710096/>
60. 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 from: <https://pubmed.ncbi.nlm.nih.gov/11286449/>
61. 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 from: <https://pubmed.ncbi.nlm.nih.gov/21255047/>
62. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. *Chem Biol Interact*. 2019 Aug 1;308:206–15.
63. 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.