JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH

RESEARCH ARTICLE

Anti Cancer Activity of Cassia Oleoresin Against Liver Cancer Cell Lines

P. HARINI[1], ANITHA ROY[2*], S. RAGHUNANDHAKUMAR[3], P. ELUMALAI[4], LAKSHMI T[5]

[1]*Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai- 600077,Tamil Nadu, India, Email : 151901095.sdc@saveetha.com*

[2]*Professor, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai-600077,Tamil Nadu, India, Email: anitharoy@saveetha.com*

[3]*Associate Professor, Cancer and Stem Cell Research Laboratory, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, No.162, PH Road, Chennai-600 077,Tamil Nadu, India, Email: raghunandhakumars.sdc@saveetha.com*

[4]*Associate Professor, Department of Pharmacology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha UniversityChennai-77,Tamil Nadu, India, Email: elumalaip.sdc@saveetha.com*

[5]*Professor, Department of Pharmacology, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha UniversityChennai-77,Tamil Nadu, India, Email: lakshmi@saveetha.com*

**Corresponding author*

ABSTRACT

Introduction: Cancer is an uncontrollable disease which destroys our body tissues rapidly and it is the most leading cause of death all over the world. Cassia oleoresin is an Indian traditional plant which is commonly used as a spice. It is used to prevent many diseases without causing any side effects. It has proven to have anticancer activity against liver cancer cell lines. The present study was designed to evaluate the anticancer activity of Cassia oleoresin against liver cancer cell lines.

Materials and methods: Cassia oleoresin (product number 4010000195) was obtained from Synthite Valley Private Limited. MTT assay was used to check the cell viability against liver cancer cell line which was treated with increasing concentration of Cassia oleoresin extract for 24 hours. The result was interpreted using SPSS software correlated using One Way ANOVA.

Results: Increase in the concentration of the Cassia oleoresin extract shows decrease in the percentage of cell viability which proves the significant anticancer activity. The cell viability was 20% at 200 µg/ml concentration of extract. The IC50 value was 65 µg/ml.

Conclusion: The extract of cassia oleoresin demonstrated potent anticancer activity against liver cancer cell lines. Hence, it can be used for treating liver cancer after evaluating the practical applicability and clinical efficacy of the drug.

KEYWORDS:
Cassia oleoresin, Liver cancer cell line, Anticancer activity, Cell viability, new approach

ARTICLE HISTORY:
Received Oct 08, 2021
Accepted Nov 15, 2021
Published Dec 21, 2021

DOI:
10.5455/jcmr.2021.12.04.04

VOLUME: 12
ISSUE: 4
ISSN: 2146-8397

INTRODUCTION

Development of cancer is registered all over the world. There are many drugs used in the treatment of cancer. Those novel drugs are toxic to cells. They not only affect the cancerous cell they also have harmful effects on the normal cells. Anticancer drugs are said to exhibit high levels of toxicity to normal cells. In traditional folk medicine natural sources like plants are said to possess health benefits (1). Herbal medicines are mostly prefered because they are very safe, non-toxic and easily available. Due to its various health properties herbs are believed to neutralize the effect of many diseases. Metabolites extracted from the plant material induce apoptosis and kill the cancer cells (2). Cancer is considered as a frightful disease which is the biggest health care issue in recent days in the human race. Plants act as reservoirs of novel chemicals for various diseases. Plants and plant products are considered as a proactive strategy for curing many diseases. It is contemplated as a revolutionizing field which is simple, safe, eco-friendly, cost effective, fast and less toxic when compared to the conventional treatment methods. Cancer is a metabolic syndrome and carcinogenesis is a complex phenomena and acts

as an signaling cascade (3).

Cinnamon is derived from the Greek word which means sweet wood originated from the inner bark of tropical evergreen cinnamon trees. It belongs to a family Lauraceae which is most commonly used as a spice. Cassia is the most commonly used type of cinnamon which is frequently used in China, Indonesia and Vietnam. It is used as a roll and in dried form which is tubular in shape referred to as a quill or cinnamon stick. It is used in food preparations in most part of the world. In ancient times cinnamon was used as a traditional medicine because of its antioxidant, antibacterial, antipyretic and anti inflammatory properties. It helps in tissue repair and it is an herbal remedy for the treatment of common cold, cardiovascular diseases, chronic gastrointestinal and gynecological disorders. It is an oriental herbal medicine for sore throats, cough, indigestion, abdominal cramps, intestinal spasms, nausea, flatulence and diarrhea. It blocks vascular endothelial growth factor and inhibits angiogenesis (4). Hepatocellular carcinoma is the most common type of heterogeneous aggressive tumour. This can be cured and treated by cinnamon before it reaches the advanced stages (5).

In recent times greater interest is shown in opportunity and herbal pills for the remedy of diverse diseases (6). Herbal medicines are said to have low side effects and these medicines are more affordable and accessible when compared to conventional medicines. In the future, it can be formulated to be used as an alternative drug by replacing the novel drug used in recent days. Medicines prepared using chemical methods are considered to be hazardous because of their high toxicity. Hence green synthesis of these herbs are more beneficial than chemical and physical approaches because of its low toxicity, biocompatibility, eco-friendliness, low energy consumption,(7,8)-(9). The aim of the study was to assess the anticancer activity of Cassia oleoresin against liver cancer cell lines.

MATERIALS AND METHODS

Study setting

Cancer and Stem Cell Research Laboratory Blue lab - Saveetha Dental College, Chennai.India

Ethical approval number

IHEC/SDC/UG-1995/21/89.

Reagents and extracts

0.25% Trypsin-EDTA solution, DMEM medium, bovine serum albumin (BSA), sodium bicarbonate solution, MTT and low melting agarose were from Sigma Chemicals Co., St. Louis, USA. Fetal bovine serum (FBS) and antimycotic solution, DMSO, were from Himedia. Sodium phosphate monobasic and dibasic, sodium hydroxide, sodium chloride, sodium carbonate, hydrochloric acid and methanol were from Sisco Research Laboratories (SRL) India.

Cell culture

The HepG2 cells were cultured in DMEM supplemented with 1% physiological saline and 10% phosphate buffer. Cell cultures were maintained at 37° C in an humidified atmosphere containing 5% CO2

Cell treatment

Cassia oleoresin was dissolved in 0.1% DMSO. HepG2 cells were plated at 1.2 X 104 cells/cm2. Twenty-four hours later, cells were fed with fresh expansion culture medium supplemented with different final concentrations of extract (10, 20, 40, 80, 120 and 200 µg/ml) or the corresponding volumes of the vehicle. After 24 h of treatment, cells were collected after 0.05% trypsin application. Cell viability was also evaluated by MTT assay.

MTT Assay

In vitro cytotoxicity assay: The effect of Cassia Oleoresin on cell viability was measured by MTT assay following the method by Mosmann. Briefly, the cells (1 × 105 cells per ml) were seeded in a 96 well microtiter plate (100 µl per well) with replications. Treatment was conducted for 24 hours with different concentrations (25, 50, 75, 100, 150, 200 µg/ml) of Cassia Oleoresin. After incubation, 20 µl of 5 mg/ml MTT stock solution was added to each well and incubated for 4 h at 37 °C. The obtained formazan crystals were solubilised with DMSO and the absorbance was measured at 570 nm using a microplate reader (SpectraMax M5, Molecular Devices, USA). Cell viability (%) has been shown as a ratio of absorbance (A570) in treated cells to absorbance in control cells (0.1 % DMSO) (A570). The IC50 was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO-treated control. Percent cell viability was calculated following the equation:

Cell viability (%) = {A570od of (sample)/A570 od of (control)} x 100

Morphological Study

Based on MTT assay we selected the IC50 value of Cassia oleoresin for further studies. The characterisation of morphological changes in liver cancer cells before and after treatment with Cassia oleoresin were observed under phase contrast microscope.

Statistical Analysis

All data obtained were analyzed and represented as mean ± SE .The results were computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. In all tests, the level of statistical significance was set at $p<0.05$.

RESULTS

In the present study we evaluated the anticancer activity of Cassia oleoresin extract on liver cancer cell line by MTT assay. MTT assay was used for quantitative assessment of viable cells

which had been adapted to measure the growth modulation of cells in vitro. The assay provides a quick, simple and cost efficient way for testing the anticancer activity of Cassia oleoresin. In this study liver cancer cell lines were treated with increasing concentrations of the extract of Cassia oleoresin for 24 hours. Cassia oleoresin extract caused dose dependent increase in anticancer activity in the liver cancer cell line.When the concentration of the extract was increased the percentage of cell viability was decreased which depicted the significant anticancer activity of Cassia oleoresin (figure 1) Morphological changes and apoptosis of liver cancerous cells were observed using Cassia oleoresin under phase contrast microscope at 20X (figure 2).

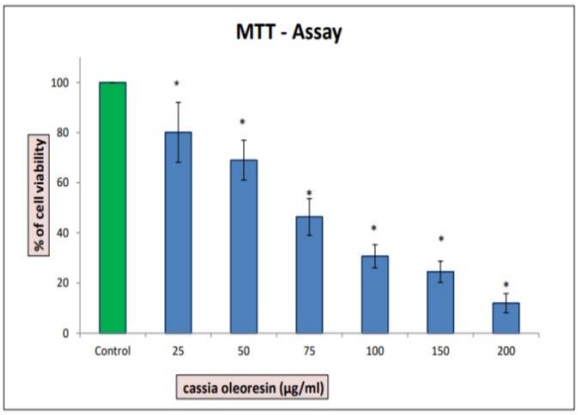
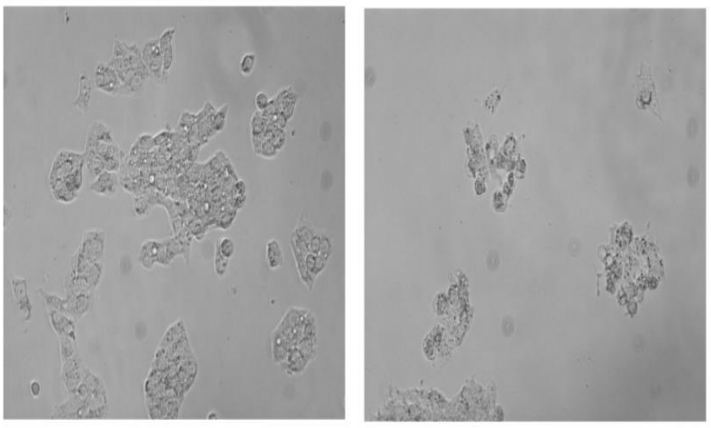


Fig.1: The graph represents the cytotoxic effects of Cassia oleoresin on HepG2 liver cancer cells. Cells were treated with Cassia oleoresin (10, 20, 40, 80, 120 and 200 µg/ml) for 24 h, and cell viability was evaluated by MTT assay. X axis represents different concentrations of cassia oleoresin and Y- axis represents the percentage of cell viability. Green colour denotes control and blue colour represents the different levels of Cassia oleoresin in 75 µg/ml. Data are shown as means ± SE (n = 3) compared with the control group with a p value of 0.009 ($p < 0.05$) was significant.



control **treated**

Fig.2: Assessment of cell morphology of HepG2 liver cancer cell line treated without and with Cassia oleoresin. Cells were treated with Cassia oleoresin (75µg/ml) for 24 h along with the control group. Images were obtained using an inverted Phase contrast microscope.

DISCUSSION

Cancer shows uncontrolled proliferation of cells. Tumour suppressor protein and oncogenic proteins express dysregulation which is the cause of cancer. Treating cancer is a challenging task particularly liver cancer is noted for its aggressive nature. Chemotherapy and radiotherapy is a general treatment and management of curing cancer (10). Plant products and its derivatives can be formulated for preparing medicines with minimal toxicity and superior efficacy. They are more reliable and cheaper to the human body. Many herbal products like Andrographis paniculata, Caralluma fimbriata have been evaluated to treat cancer (11),(12).

In previous studies it is reported that Cinnamon inhibits the growth of tumor cells. It decreases the expression of COX-2 and HIF-1α in tumour tissues (13). In present study it is reported that Cassia oleoresin has a potent anti cancer activity against liver cancer cell line. When the concentration of the extract increased the percentage of cell viability was decreased. That is at 200 µg/ml of Cassia oleoresin the cell viability is only 20 % which indicates the powerful activity of the extract against the liver cancer cells. In a previous study done using Cassia

oleoresin to check the cytotoxic effect it is found that even a low dose of extract showed significant activity. They stated that it can be formulated using nanoparticles for curing many diseases (14). In a present study it is reported that the extract showed potent anticancer activity against liver cancer cell lines.

To determine the extent of cell viability MTT assay was used which is a reliable assay. In previous studies the extract showed the induction of apoptosis in human hepatoma cancer cells which indicates the anticancer activity (15–19) (20). Previous studies concluded that the application of the cinnamon extract or the active compounds of cinnamon inhibits the development of cancer at various stages from initiation to metastasis. Cinnamon prevents the onset of cancer or delays its process (21). Some studies suggested that Cassia Inhibits the viability, survival and proliferation of tumor cells without having harmful effects on the normal cells. The extract has the ability to induce apoptosis in tumor cells and it also inhibits the NFkB activity. Cassia are said to impede the activities of proangiogenic factors which are responsible for the tumor cells to proliferate and it also increases the activity of CD8(+) T cells (22). Similarly, in the present study also reported that Cassia oleoresin suppresses the activity of cancer cells and disrupts its morphological characters which indicates its potent activity.

The extract of Cinnamon inhibits the angiogenesis involved in cancer. 2'hydroxycinnamaldehyde is a chemical derived from the Cinnamon that inhibits the growth of tumor cells. The activity is also due to the presence of chemical and volatile compounds present in the extract (23). In previous studies it is reported that Cinnamon inhibits the activity of NF-kB, TNF-α and IL-8 which acts as a potential anti cancer agent (24). There are some potential limitations, that is the study is taken into consideration with only one herb and it should be considered to be done on a large scale and there is a high possibility of occurrence of error. Our team has extensive knowledge and research experience that has translated into high quality publications (25–29) (30) . The study was carried out in-vitro and it cannot be assumed that the result of anticancer activity could be translated into clinical effectiveness which proves to be a limitation. In future clinical trials and animal experiments can be done to check the toxicity of the extract. In the future it can be formulated as an alternative drug and commercial products can be prepared which will possess a great and potential value in the herbal markets.

CONCLUSION

Cinnamon has been used as a spice in our daily life without any side effects. Several reports have dealt with the numerous properties of Cinnamon in the forms of bark, essential oils, bark powder, phenolic compounds, flavonoids and isolated components.(31-40). Each of these properties plays a key role in the advancement of human health. From the present study it is evident that Cinnamon possesses a good anticancer activity. The significant health benefits of cinnamon have been explored. Further investigations are necessary to provide additional clinical evidence against the activity of cancer.

ACKNOWLEDGEMENT

The authors express their gratitude to Synthite Industries Private Limited, Kerala for providing Cassia oleoresin to carry out this research study and thanking SKR Pack Tech Private Limited, Thrivallur, Tamilnadu for providing support to conduct the research.

CONFLICT OF INTEREST

NIL

SOURCE OF FUNDING

SKR Pack Tech Pvt Limited,Thiruvallur, Tamil Nadu

REFERENCES

1. Lichota A, Gwozdzinski K. Anticancer Activity of Natural Compounds from Plant and Marine Environment. Int J Mol Sci [Internet]. 2018 Nov 9;19(11). Available from: http://dx.doi.org/10.3390/ijms19113533
2. Schwarzenberg K von, von Schwarzenberg K, Vollmar AM. Targeting apoptosis pathways by natural compounds in cancer: Marine compounds as lead structures and chemical tools for cancer therapy [Internet]. Vol. 332, Cancer Letters. 2013. p. 295–303. Available from: http://dx.doi.org/10.1016/j.canlet.2010.07.004
3. Khan I, Zaib S, Javed M, Rashid F, Iqbal J, Ibrar A. Antiproliferative and Pro-Apoptotic Effects of Thiazolo[3,2–b][1,2,4]triazoles in Breast and Cervical Cancer Cells [Internet]. Vol. 21, Anti-Cancer Agents in Medicinal Chemistry. 2021. Available from: http://dx.doi.org/10.2174/1871520621666210126092303
4. Panickar K, Cao H, Qin B, Anderson RA. Molecular Targets and Health Benefits of Cinnamon [Internet]. Molecular Targets and Therapeutic Uses of Spices. 2009. p. 87–115. Available from: http://dx.doi.org/10.1142/9789812837912_0004
5. Aly SM, Fetaih HA, Hassanin AAI, Abomughaid MM, Ismail AA. Protective Effects of Garlic and Cinnamon Oils on Hepatocellular Carcinoma in Albino Rats. Anal Cell Pathol . 2019 Oct 22;2019:9895485.
6. Serhan CN, Ward PA, Gilroy DW. Fundamentals of Inflammation. Cambridge University Press; 2010. 473 p.
7. A MTAK, Mohamed Thamemul Ansari K, Roy A, Rajeshkumar S. Anti-inflammatory activity of cinnamon oil mediated silver nanoparticles -An in vitro study [Internet]. Vol. 10, International Journal of Research in Pharmaceutical Sciences. 2019. p. 2970–2. Available from: http://dx.doi.org/10.26452/ijrps.v10i4.1579
8. S. RJ, Roy A, Shanmugam R, E. DW. Preparation and Characterization of Cinnamon Oil Mediated Gold Nanoparticles and Evaluation of Its Cytotoxicity Using Brine Shrimp LethalityAssay [Internet]. Vol. 9, Journal of Evolution of Medical and Dental Sciences. 2020. p. 2894–7. Available from: http://dx.doi.org/10.14260/jemds/2020/633
9. Anitha R, Prathoshni S, Lakshmi T. The effect of capsicum oleoresin on nitric oxide production and nitric oxide synthase gene expression in macrophage cell line [Internet]. Vol. 10,

Pharmacognosy Research. 2018. p. 343. Available from: http://dx.doi.org/10.4103/pr.pr_46_18

10. M.p I, Induja MP, Saveetha Dental College and Hospitals, Road PH, Chennai, Gayathri R, et al. Cytotoxicity of walnut oil on oral cancer cell lines [Internet]. Vol. 6, International Journal of Current Advanced Research. 2017. p. 3175–6. Available from: http://dx.doi.org/10.24327/ijcar.2017.3176.0216
11. Roy A, Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, Nadu- T, et al. Molecular docking analysis of compounds from Andrographis paniculata with EGFR [Internet]. Vol. 17, Bioinformation. 2021. p. 23–8. Available from: http://dx.doi.org/10.6026/97320630017023
12. Suhasini SJ, Jennifer Suhasini S, Roy A, Sosa G, Lakshmi T. The Cytotoxic effect of Caralluma fimbriata on KB cell lines [Internet]. Vol. 12, Research Journal of Pharmacy and Technology. 2019. p. 4995. Available from: http://dx.doi.org/10.5958/0974-360x.2019.00865.5
13. Hamidpour R, Hamidpour M, Hamidpour S, Shahlari M. Cinnamon from the selection of traditional applications to its novel effects on the inhibition of angiogenesis in cancer cells and prevention of Alzheimer's disease, and a series of functions such as antioxidant, anticholesterol, antidiabetes, antibacterial, antifungal, nematicidal, acaracidal, and repellent activities [Internet]. Vol. 5, Journal of Traditional and Complementary Medicine. 2015. p. 66–70. Available from: http://dx.doi.org/10.1016/j.jtcme.2014.11.008
14. Anjana G, Roy A, Rajeshkumar S, Ezhilarasan D. Cassia oleoresin Mediated Synthesis of Magnesium Oxide Nanoparticles and Brine Shrimp Lethality Assay [Internet]. Journal of Pharmaceutical Research International. 2020. p. 75–82. Available from: http://dx.doi.org/10.9734/jpri/2020/v32i1530628
15. Koka P, Mundre RS, Rangarajan R, Chandramohan Y, Subramanian RK, Dhanasekaran A. Uncoupling Warburg effect and stemness in CD133 cancer stem cells from Saos-2 (osteosarcoma) cell line under hypoxia. Mol Biol Rep. 2018 Dec;45(6):1653–62.
16. Raghunandhakumar S, Paramasivam A, Senthilraja S, Naveenkumar C, Asokkumar S, Binuclara J, et al. Thymoquinone inhibits cell proliferation through regulation of G1/S phase cell cycle transition in N-nitrosodiethylamine-induced experimental rat hepatocellular carcinoma. Toxicol Lett. 2013 Oct 23;223(1):60–72.
17. Asokkumar S, Naveenkumar C, Raghunandhakumar S, Kamaraj S, Anandakumar P, Jagan S, et al. Antiproliferative and antioxidant potential of beta-ionone against benzo(a)pyrene-induced lung carcinogenesis in Swiss albino mice. Mol Cell Biochem. 2012 Apr;363(1-2):335–45.
18. Anandakumar P, Kamaraj S, Jagan S, Ramakrishnan G, Asokkumar S, Naveenkumar C, et al. Capsaicin inhibits benzo(a)pyrene-induced lung carcinogenesis in an in vivo mouse model. Inflamm Res. 2012 Nov;61(11):1169–75.
19. Paramasivam A, Raghunandhakumar S, Priyadharsini JV, Jayaraman G. In Vitro Anti-Neuroblastoma Activity of Thymoquinone Against Neuro-2a Cells via Cell-cycle Arrest. Asian Pac J Cancer Prev. 2015;16(18):8313–9.
20. Nugraha AT, Department of Pharmacy, Universities Islam Indonesia, Yogyakarta, Ramadhan V, Pandapotan H, et al. A Study of Proliferative Activity of Herbs Eriocaulon Cinereum R.Br on Cervical Cancer Cells (HeLa) with MTT Assay Method [Internet]. Vol. 6, International Journal of Pharma Medicine and Biological Sciences. 2017. p. 73–6. Available from: http://dx.doi.org/10.18178/ijpmbs.6.2.73-76
21. Ravindran PN, Nirmal-Babu K, Shylaja M. Cinnamon and Cassia: The Genus Cinnamomum. CRC Press; 2003. 384 p.
22. Kawatra P, Rajagopalan R. Cinnamon: Mystic powers of a minute ingredient. Pharmacognosy Res. 2015 Jun;7(Suppl 1):S1–6.
23. Rao PV, Gan SH. Cinnamon: a multifaceted medicinal plant. Evid Based Complement Alternat Med. 2014 Apr 10;2014:642942.
24. Cabello CM, Lamore SD, Bair WB 3rd, Davis AL, Azimian SM, Wondrak GT. DCPIP (2,6-dichlorophenolindophenol) as a genotype-directed redox chemotherapeutic targeting NQO1*2 breast carcinoma. Free Radic Res. 2011 Mar;45(3):276–92.
25. 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.
26. 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.
27. 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.
28. 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
29. 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.
30. Website [Internet]. Available from: Veerasamy, R.; Roy, A.; Karunakaran, R.; Rajak, H. Structure–Activity Relationship Analysis of Benzimidazoles as Emerging Anti-Inflammatory Agents: An Overview. Pharmaceuticals 2021, 14, 663. https://doi.org/10.3390/ ph14070663
31. 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.
32. 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/
33. 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/
34. 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/
35. 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/

36. 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/

37. 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/

38. 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/

39. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact. 2019 Aug 1;308:206–15.

40. 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.

42. Veerasamy, R, Roy, A, Karunakaran, R, Rajak, H. Structure-Activity Relationship Analysis of Benzimidazoles as Emerging Anti-Inflammatory Agents: An Overview. Pharmaceuticals 2021, 14, 663. https://doi.org/10.3390/ ph14070663