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RESEARCH ARTICLE
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Evaluation Of Antimicrobial And Cytotoxic Effects Of 1,25 Dihydroxycholecalciferol Formulation- An Invitro Study

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ABSTRACT

Background: Vitamin D is a fat-soluble pro-hormone that can be obtained through exposure to the sun or derived from ingested foods or supplements. It is available in two forms: vitamin D2, also known as ergocalciferol, and vitamin D3, also known as cholecalciferol. It is important to remember, however, that many individuals may have inadequate exposure to UV light due to lack of outdoor activity and the use of protective sunscreen lotions and clothing

Aim: To prepare a formulation containing 1,25 dihydroxycholecalciferol. To determine the antimicrobial and cytotoxic effects of the formulation containing 1,25 dihydroxycholecalciferol .

Materials And Methods: In the present study, preparation of the formulation was carried out initially and then the antimicrobial and cytotoxic activity of the formulation was assessed. The antimicrobial activity of the mouthwash formulation was carried out using agar well diffusion method. Different concentrations of nanoparticles were tested against Streptococcus mutans, E.faecalis lactobacillus and Candida albicans. The cytoxicity activity of the mouthwash was assessed using brine shrimp lethality assay method.

Results: The cytotoxic activity was found to be better at low concentration.8 nauplii survived at 5 µL, 10 µL, 20 µL after 24 hours.The antimicrobial activity of the mouthwash formulation showed that the mouthwash formulation had the highest zone of inhibhition seen in 100 µL concentration on C.albicans almost similar to the contrl antibiotic in comparison to the oral pathogens.

Discussion:1,25 dihydroxycholecalciferol has many medicinal properties. Our study has evaluated the concentration of the formulation and has found positive results.

Conclusion: The prepared formulation had better antimicrobial activity and exhibhited less cytotxicity at lower concentrations.

Keywords: *1,25 dihydroxycholecalciferol, vitamin D, formulation, antioxidant, anti-inflammatory*

INTRODUCTION

Vitamin D is a fat-soluble pro-hormone that can be obtained through exposure to the sun or derived from ingested foods or supplements. It is available in two forms: vitamin D2, also known as ergocalciferol, and vitamin D3, also known as cholecalciferol.[1-3] These formulations differ chemically only in their side-chain structure. Vitamin D2 is most commonly manufactured through the process of ultraviolet (UV) irradiation of ergosterol extracted from yeast, whereas vitamin D3 is manufactured synthetically by the irradiation of 7-dehydrocholesterol extracted from lanolin. Regardless of how vitamin D is manufactured or acquired, these compounds have weak biological activity in their initial form and require enzymatic conversion to produce the more active metabolites that are best used by the body. This occurs through an enzymatic process that starts in the liver, where vitamin D is converted to 25-hydroxyvitamin D [25(OH)D], its major circulating and storage form. This product is then converted to 1,25-dihydroxyvitamin D [1,25(OH)2D], its hormonally active form, by enzymes as it travels through the kidneys. [4-7]

Synthesis that begins in the skin is the major natural source of vitamin D. Previtamin D3 is synthesized in the two innermost layers of the epidermis, the stratum basale and the stratum spinosum, from 7-dehydrocholesterol during exposure to UV light (UVB type) at wavelengths between 270 nm and 300 nm. These wavelengths are present in sunlight and in light emitted by UV lamps in tanning beds. Previtamin D3 undergoes a temperature-dependent spontaneous isomerisation to form vitamin D3. [8-10]

The length of daily exposure required to obtain the sunlight equivalent of oral vitamin D supplementation is difficult to predict on an individual basis and varies based on skin type, amount of skin exposed, latitude, season, and time of day. Nevertheless, 15 to 30 minutes of unprotected sun exposure two to four times a week is generally recommended to maintain adequate levels of vitamin D. It is important to remember, however, that many individuals may have inadequate exposure to UV light due to lack of outdoor activity and the use of protective sunscreen lotions and clothing. In addition, at northern latitudes, there may not be sufficient UV radiation to synthesize vitamin D, particularly during the winter months. For these reasons, at least in the United States, milk, infant formula, breakfast cereals, and other select foods are fortified with vitamin D. [11] The aim of the present study is to prepare a formulation containing 1,25 dihydroxycholecalciferol and to determine the antioxidant and anti-inflammatory properties of the formulation at various concentrations.

MATERIALS AND METHODS

Mouthwash Formulation

1g of each F.benghalensis,A.indica, M.piperita extract was taken in a beaker.The extract was then mixed with 100 ml of distilled water.The prepared mixture was then subjected to 15 minutes of constant boiling.An Extract was obtained which was then filtered to 50-60 ml

to get the final extract.(Fig.1-3)

Cytotoxicity Activity Testing

Cytotoxicity activity of the prepared mouthwash formulation was done using Brine shrimp lethality assay.

Antimicrobial Effect Testing

Antibacterial Activity

Antibacterial activity of the formulation was tested against the strain staphylococcus aureus, Bacillus, and E.coli. MHA agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar were prepared and sterilize for 45 minutes at 120lbs. Media poured into the sterilized plates and let stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed.The formulation with different concentration were loaded and the plates were incubated for 24 hours at 37 ° C. After the incubation time the zone of inhibition were measured. [16-21]

Antifungal activity

Aspergillus fumigates, Aspergillus flavus, Aspergillus niger are used as test pathogens by agar well diffusion assay.Sabouraud's Dextrose Agar is used to prepare the medium.The prepared and sterilized medium was swabbed with test organisms and nanoparticles with different concentration were added to the wells.The plates were incubated at 28° C for 48-72hours. After the incubation time the zone of inhibition were measured.[22,23](Fig.4)

Brine Shrimp Lethality Assay

Salt water preparation

2g of iodine free salt was weighed and dissolved in 200ml of distilled water.

6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20µL,40 µL,60 µL.80 µL,100 µL). Then the formulation were added according to the concentration level. The plates were incubated for 24 hours.[24-27]. After 24 hours, the ELISA plates were observed and noted for number of live nauplii's present and calculated by using following formula, number of dead nauplii/number of dead nauplii+number of live nauplii×100 (fig.5)

RESULTS & DISCUSSION

Antimicrobial activity

The antimicrobial activity of the formulation containing 1,25 dihydroxycholecalciferol is depicted in figure 6, 7, 8 and 9 .The effects of formulation were tested for the antimicrobial activity against Streptococcus mutans, Lactobacillus ,E.faecalis and Candida albicans. The formulation showed a very small zone of inhibition against S. mutans ,S.aureus and E.faecalis showed 12mm, 14, 15 mm at 100µL concentration. The maximum zone of inhibition for C.albicans is 11 mm at 100 µL concentration and The antibacterial effect of formulation on microbes have the ability to attach to the bacterial cell and eventually penetrate into it thus causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. [16-20]The antimicrobial activity of the formulation showed that the formulation had the highest zone of inhibition seen in 100 µL concentration on C.albicans almost similar to the control antibiotic in comparison to the oral pathogens.(Fig.6-9)

Cytotoxicity Activity

The cytotoxic activity of formulation was assessed by using Brine shrimp Lethality test. The prepared formulation did not show cytotoxic activity on brine shrimp as all the shrimps in the 6 wells in which the extract was added survived on the first day. From the brine shrimp lethality test done, it was noted that on the first day all the nauplii survived, and on the second day 8 nauplii survived at lower concentrations. As far as the concentration of the formulation, the cytotoxicity was found to be better at lower concentrations where 8 nauplii survived. Hence from the current study, it was noted that the lower concentrations can be used for biomedical applications. (Fig.10)

DISCUSSION

Vitamin D (also referred to as "1,25 dihydroxycholecalciferol") is a fat-soluble vitamin that is naturally present in a few foods, added to others, and available as a dietary supplement. It is also produced endogenously when ultraviolet (UV) rays from sunlight strike the skin and trigger vitamin D synthesis. Vitamin D obtained from sun exposure, foods, and supplements is biologically inert and must undergo two hydroxylations in the body for activation. The first hydroxylation, which occurs in the liver, converts vitamin D to 25-hydroxyvitamin D [25(OH)D], also known as "calcidiol." The second hydroxylation occurs primarily in the kidney and forms the physiologically active 1,25-dihydroxyvitamin D [1,25(OH)2D], also known as "calcitriol" [12].

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal bone mineralization and to prevent hypocalcemic tetany (involuntary contraction of muscles, leading to cramps and spasms). It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts [1-3]. Without sufficient vitamin D, bones can become thin, brittle, or misshapen. Vitamin D sufficiency prevents rickets in children and osteomalacia in

adults. Together with calcium, vitamin D also helps protect older adults from osteoporosis.[13]

Vitamin D has other roles in the body, including reduction of inflammation as well as modulation of such processes as cell growth, neuromuscular and immune function, and glucose metabolism [14-15]. Many genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis are modulated in part by vitamin D. Many tissues have vitamin D receptors, and some convert 25(OH)D to 1,25(OH)2D.

The role of Vitamin D has been gaining attention in oral health. Deficiency of Vitamin D is associated with defective tooth mineralisation resulting in enamel and dentin defects. This in turn increases the risk of a tooth to dental caries. There are 2 mechanisms by which Vitamin D deficiency affects tooth mineralisation leading to caries. Severe Vitamin D (<10ng/mL) deficiency causes hypocalcaemia and hypophosphatemia leading to secondary hyperparathyroidism. Hyperparathyroidism promotes Calcium absorption and renal production of 1α, 25-dihydroxyvitamin D thereby elevating serum levels of calcium and decreasing levels of inorganic phosphate. This leads to the loss of Vitamin D signalling pathways in cells of tooth causing defective mineralisation [16,17].

Our study is first of its kind to evaluate the effective concentration of 1,25 dihydroxycholecalciferol for supplementation. The cytotoxic activity was found to be better at low concentration. The antimicrobial activity of the formulation showed that the formulation had the highest zone of inhibition seen in 100 µL concentration on C.albicans almost similar to the control antibiotic in comparison to the oral pathogens

CONCLUSION

1,25 dihydroxycholecalciferol formulation has many medicinal properties. Our study has evaluated the various concentration of this formulation and has found positive results. The cytotoxic activity was found to be better at low concentration. 8 nauplii survived at 5 µL, 10 µL, 20 µL after 24 hours. The antimicrobial activity of the formulation showed that the formulation had the highest zone of inhibition seen in 100 µL concentration on C.albicans almost similar to the control antibiotic in comparison to the oral pathogens. So the formulation can be used for various biomedical application with lower concentrations.

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FIG 1: Preparation of 1,25 dihydroxycholecalciferol formulation

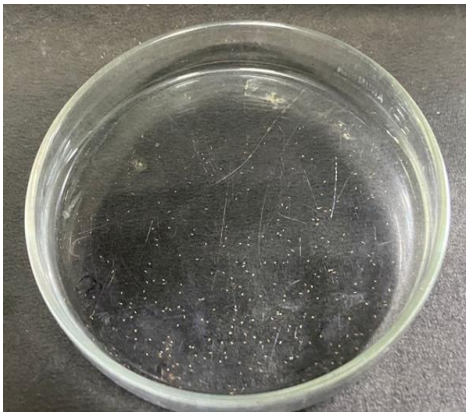

FIG 2: Isolation of naupliis for BSLA

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FIG 3: Brine Shrimp Lethality Assay (BSLA)

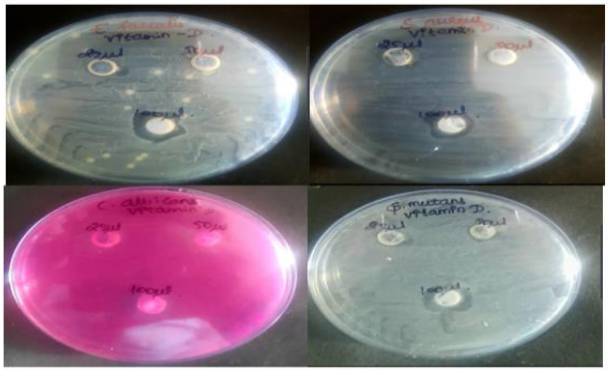

FIG 4: Antimicrobial activity exhibited by 1,25 dihydroxycholecalciferol formulation at various concentration

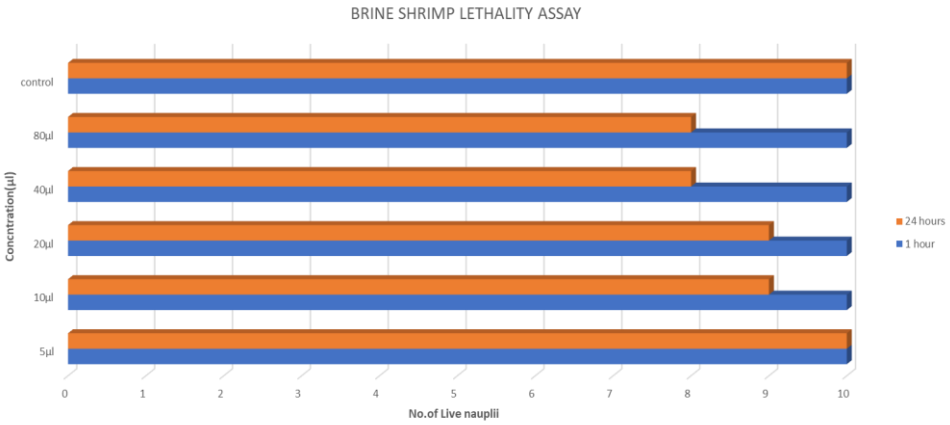

FIG 5: Graph showing Cytotoxicity activity of 1,25 dihydroxycholecalciferol formulation

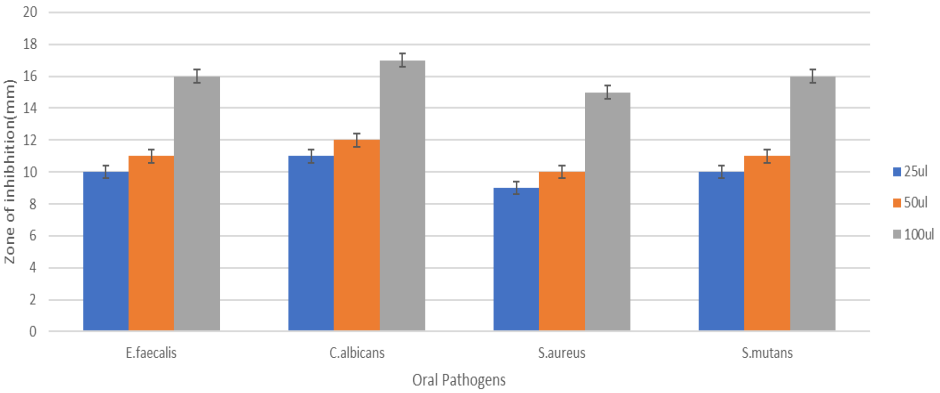

FIG 6: Graph showing Antimicrobial activity of 1,25 dihydroxycholecalciferol formulation

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