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# Evaluation Of Antioxidant and Anti-Inflammatory Effects Of 1,25 Dihydroxycholecalciferol Formulation- An Invitro Study

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## ABSTRACT

**Background:** Vitamin D is a fat-soluble pro-hormone that is mainly obtained through the exposure to sun. Vitamin D is present mainly in two forms: vitamin D2, also known as ergocalciferol, and vitamin D3, also known as cholecalciferol. Vitamin D plays an essential role in maintaining bone metabolism. Due to lifestyle modifications, there is a worldwide reported cases of vitamin D deficiency due to inadequate sun exposure and lack of intake of foods rich in Vitamin D.

**Aim:** To prepare a formulation containing 1,25 dihydroxycholecalciferol. To determine the antioxidant and anti-inflammatory 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 antioxidant and anti-inflammatory activity of the formulation was assessed. The antioxidant activity of the formulation was carried out using DPPH Assay method and Hydroxyl Radical Scavenging Assay method. The anti-inflamnatory activity of the formulation was assessed using albumin denaturation assay method and egg albumin denaturation assay method.

**Results:** The antioxidant and anti-inflammatory activity was found to be better at low concentration. At concentrations of 10-30µl, the antioxidant activity of the prepared formulation was similar to that of the standard. The anti-inflammatory activity was found to be highest at 10 µl concentration.

Conclusion: The prepared formulation had better antioxidant and anti-inflammatory activity at lower concentrations.

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

## INTRODUCTION

Vitamin D is a fat-soluble pro-hormone that is mainly obtained through exposure to the sun or derived from ingested foods or supplements. It is mainly available in two forms: vitamin $D_2$, also known as ergocalciferol, and vitamin $D_3$, also known as cholecalciferol.[1,2] These formulations differ chemically only in their side-chain structure. Vitamin $D_2$ is most commonly manufactured through the process of ultraviolet (UV) irradiation of ergosterol extracted from yeast, whereas vitamin $D_3$ 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.[3,4] 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.

Synthesis that begins in the skin is the major natural source of vitamin D. [5,6,7] Previtamin $D_3$ 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 $D_3$ undergoes a temperature-dependent spontaneous isomerisation to form vitamin $D_3$. [8,9]

Due to lifestyle changes , Vitamin D3 Deficiency has been reported worldwide. Vitamin D is essential for bone metabolism. It stimulates calcium and phosphate absorption by the intestine, regulates bone metabolism, and negatively controls PTH secretion through the endocrine action of its active metabolite calcitriol. [10,11]Vitamin D also plays an important role in the regulation of arterial blood pressure and the prevention of cardiovascular complications, modulation of immunological responses, regulation of insulin production and prevention against diabetes, protection against certain cancers, renoprotection, and other beneficial actions.[12,13] Vitamin D supplementation has been recommended for patients with Vitamin D deficiency. Though there are various formulations of Vitamin D available, the concentration at which the Vitamin D formulation is most effective is not studied. So, 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

### *Preparation Of Formulation*

1g of 1,25 dihydroxycholecalciferol powder was taken in a beaker. The mixture was then mixed with 10 ml of ethanol and 100 ml of distilled water. The prepared mixture was constantly mixed until the powder was completely dissolved in the solvent to obtain a homogenous solution. A formulation was obtained which was then stored in a vial.(Fig.1)

### *Antioxidant Activivty*

### *Dpph Method*

DPPH assay was used to assess the antioxidant activity of the formulation. Diverse concentrations (10µL,20µL,30µL,40µL,50µL) of formulation was taken and 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4)were added and incubated for 30 minutes. The absorbance of DPPH free radicals was determined at 517 nm. Ascorbic acid was used as the standard. The percentage of inhibition was determined using the formula

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

### *Hydroxyl Radical Scavenging Assay*

1.0mL of the reaction mixture (2-deoxy-2-ribose + various concentrations of the formulation +Fecl3 + H2O2+ ascorbic acid) was taken and incubated for a period of 1 hour at 37°C. The extent of deoxyribose degradation was determined at about 532nm against the blank solution. Vitamin E was used as a positive control.

***Anti-Inflammatory Activity***

***Albumin Denaturation Assay***

The anti-inflammatory activity for Solanum tarvum gel was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (Pratik Das et al.,2019). 0.05 mL of Solanum tarvum gel of various fixation (10µL,20µL,30µL,40µL,50µL)was added to 0.45 mL bovine serum albumin(1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control.

Percentage of protein denaturation was determined utilizing following equation,

% inhibition= Absorbance of control- Absorbance of sample×100

Absorbance of control

***Egg Albumin Denaturation Assay***

A 5ml solution was made which was comprised of 2.8ml of freshly prepared phosphate buffered saline of pH - 6.3, 0.2 ml of egg albumin extracted from hen's egg. Specific concentrations were prepared separately for Syzygium caryophyllatum as (10µL,20µL,30µL,40µL,50µL). Diclofenac sodium was used as the positive control. Then the mixtures were heated in water bath at 37ºC for 15 minutes. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm.

## RESULTS

***Antioxidant activity***

The antioxidant activity of the formulation containing 1,25 dihydroxycholecalciferol is depicted in figure 2,3 [Fig.2,3] The results of the DPPH assay and H2O2 assay for antioxidant activity revealed that, at higher concentrations the standard had more antioxidant activity compared to our prepared formulation. However at the concentration of 10µl, the antioxidant activity of the prepared formulation was similar to that of the standard.

***Anti-inflammatory Activity***

The results showed that the percentage of inhibition was greater in the standard when compared to the prepared formulation. [Fig.4,5] However the anti-inflammatory activity was highest at 10 µL concentration, followed by 20 µL, and least at 50 µL when compared to the standard.

From these results, it can be said that the antioxidant and anti-inflammatory activity of 1,25 dihydroxycholecalciferol was better when used at lower concentration and can be used for various biomedical applications.

## DISCUSSION

Vitamin D3 is well known for its function as a steroid hormone on skeletal tissue. In particular, it is responsible for the increase in plasma Ca and phosphate levels required for bone mineralisation, as well as for neuromuscular junction activity, vasodilatation, nerve transmission and hormone secretion. Several researchers have suggested relationships between vitamin D intake and health outcomes such as cancer prevention and increased immunity.[14,15] Others have proposed possible roles in preventing diabetes or preeclampsia during pregnancy[16,17], as well as counteracting inflammation[18,19], although the antioxidant effect of vitamin D is not uniformly proven[20]. At the same time, data suggest that a large part of the general population is vitamin D deficient, even in those countries where sun exposure is prolonged[15]

Considering human studies, several health outcomes have been associated with vitamin D plasma levels but little is known about its antioxidant effects. Different markers have been proposed to assess antioxidant status, such as glutathione peroxidase, SOD, catalase, glutathione S-transferase, or by determining total antioxidant. The present study was done to assess the most effective concentration of vitamin D formulation for its beneficial action. The Vitamin D formulation was found to have maximum antioxidant activity at lower concentration. The antioxidant activity of Vitamin D formulation

was similar to the standard at lower concentrations.

Previous studies have attempted to delineate the effects of vitamin D on inflammatory cells and processes. In vitro, vitamin D has been shown to promote monocyte differentiation to macrophages, preventing them from releasing inflammatory cytokines and reducing their ability to present antigens to lymphocytes by inhibiting cell surface expression of the class 2 major histocompatibility complex (MHC-II) molecule. Vitamin D also suppresses the proliferation and stimulatory abilities of T cells and monocytes, and downregulates proinflammatory cytokines, including C reactive protein (CRP), tumour necrosis factor α (TNFα), interleukin (IL) 6, IL-1 and IL-8, while upregulating anti-inflammatory cytokines such as IL-10.2 In vitro data has also shown associations between absence of the VDR and increased nuclear factor κB (NFκB) activity, a transcription factor with a key role in immunomodulation, and in the pathophysiology of several inflammatory diseases and chronic inflammatory states.[21,22,23]

We had assessed the various concentrations of the prepared vitamin D formulation and found it to be the most effective at lower concentrations at 10μL and 20 μL. This low concentration of Vitamin D can be used to supplement patients with Vitamin D deficiency and is found to have better antioxidant and anti-inflammatory activity almost similar to that of the standard.

***Source of funding***
Nil

***Conflict of interest***
None declared

***Declaration statement***
The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work and authors alone are responsible for the content and writing of the paper.

## CONCLUSION

Vitamin D is known to have many medicinal benefits. Due to lifestyle modifications, Vitamin D deficiency has been reported worldwide. Vitamin D supplementation is necessary to combat this world wide deficiency for effective metabolism. However the concentration at which the formulation of 1,25 dihydroxycholecalciferol is the most effective with least side effects is less understood. The present study has analysed the various concentration of Vitamin D formulation and has been found to have the maximum antioxidant and anti-inflammatory activity at 10μL concentrations.

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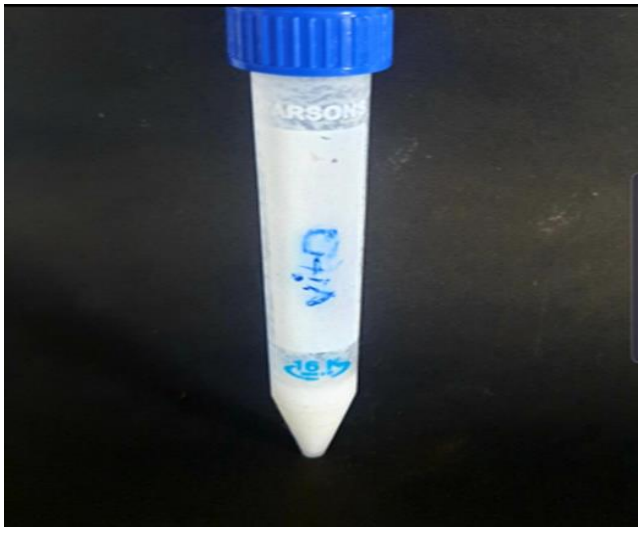

**Fig.1 Preparation of 1,25 dihydroxycholecalciferol formulation**

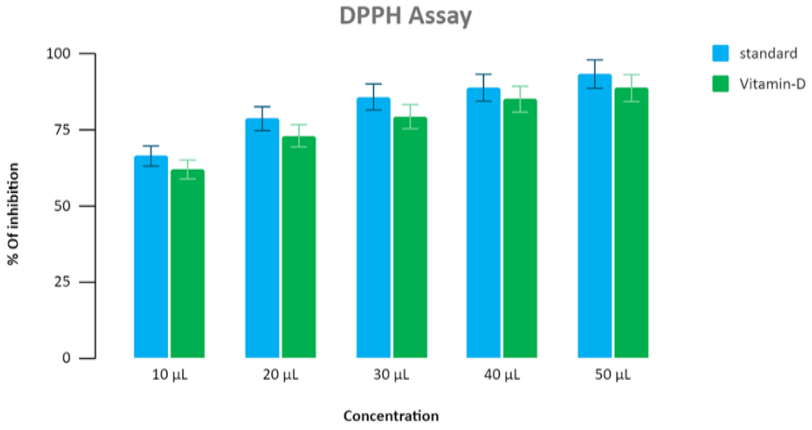


**FIG 2:** Graph showing the results of antioxidant activity of 1,25 dihydroxycholecalciferol using DPPH assay

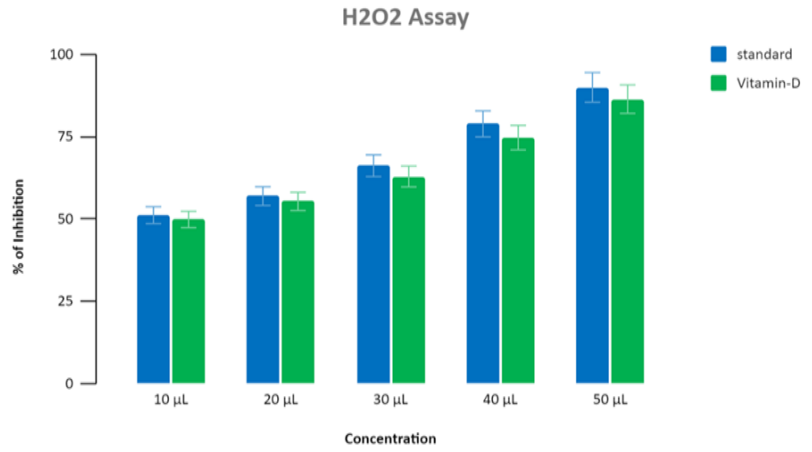


**FIG 3:** Graph showing the results of antioxidant activity of 1,25 dihydroxycholecalciferol using H2O2 assay

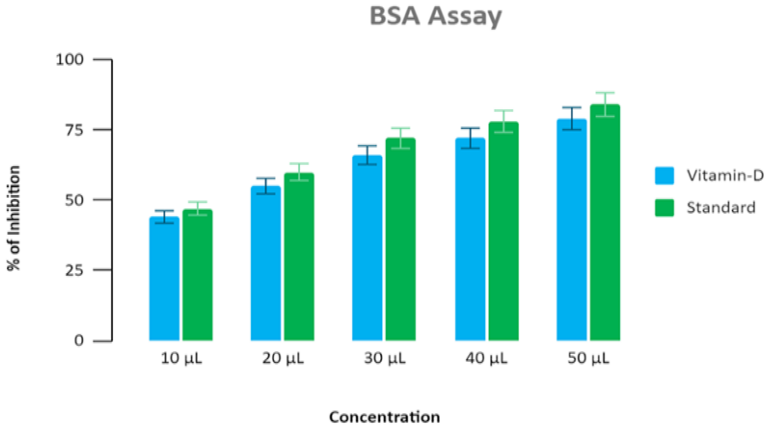


**FIG 4:** Graph showing the results of anti-inflammatory activity of 1,25 dihydroxycholecalciferol using BSA assay

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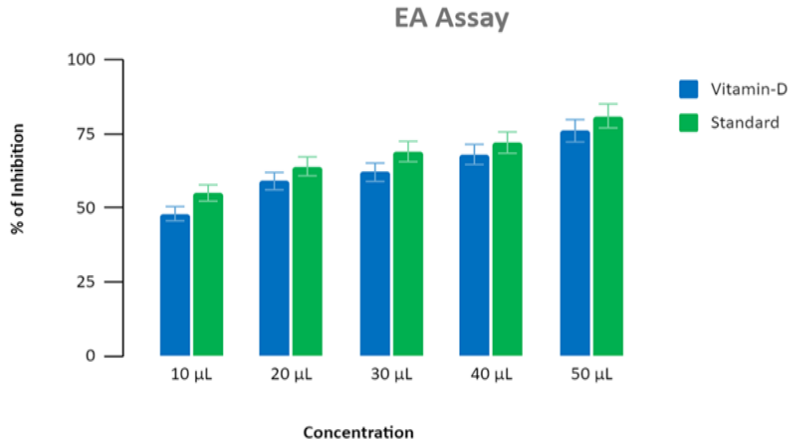


**FIG 5:** Graph showing the results of anti-inflammatory activity of 1,25 dihydroxycholecalciferol using EA assay

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