

Evaluation Of Pleurotus Florida (White Oyster Mushroom) Cultivated In Amalaki Medium In The Management Of Vitamin D Deficiency

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Abstract

Oyster mushroom (*Pleurotus florida*), known as 'dhingri' in India, grows spontaneously on decaying and dead wooden logs or on dying trunks of deciduous or coniferous trees in temperate and tropical woodlands. In addition to supplementing nutritional requirements, the cultivation of *Pleurotus* also provides an opportunity for self-employment and mycoremediation. In accordance with conventional cultivation practices *Pleurotus florida* is grown in paddy straw treated with amalaki. After cultivation, when exposed to sunlight or a UV lamp, oyster mushrooms produce nutritionally significant levels of vitamin D. The vitamin D content of UV-exposed oyster mushrooms is likely to remain at 10ug/100g fresh weight, which is greater than the vitamin D content of the majority of vitamin D-containing foods and comparable to the recommended daily intake of vitamin D at the global level. Vitamin D deficiency leads to reduced calcium absorption, which in turn leads to generalized body pains, hair fall and fatigue which are the most common complaints. Eligible candidates (n=50) were screened by computerized randomization method to receive *Pleurotus florida* soup during the 03-month study. The efficacy parameters were evaluated through the Vit D 25 (OH) blood investigation.

Keywords: Amalaki, *Pleurotus florida*, UV lamp, Vitamin D, Soup, Mushrooms.

Introduction

Vitamin D, called as the sunlight vitamin, is a fat-soluble compound with well-established antirachitic properties (1). cholecalciferol, which is Vitamin D₃, and ergocalciferol, which is Vitamin D₂, are both lipid-soluble compounds with a four-ringed cholesterol (2). Vitamin D generally refers to Vitamin D₃. Vitamin D is a naturally produced vitamin on exposure to sunlight. Approximately 90% of the necessary Vitamin D is produced by the epidermis in response to UV exposure (3). Vitamin D deficiency is becoming a worldwide epidemic (4). Vitamin D deficiency prevalence ranges from 40% to 99%, with the majority of research indicating a prevalence of 80%–90% (5). One billion people, of all ethnicities and ages, are estimated to be deficient in vitamin D. Hypovitaminosis D is an independent risk factor for overall mortality in the general population, making vitamin D deficiency a significant public health concern. Dietary supplementation of vitamin D to prevent its extreme deficiency would appear to be an essential health intervention (6). The majority of vitamin D deficiency symptoms are asymptomatic, but fatigue, insomnia, depression, hair loss, muscle weakness and discomfort, loss of appetite, etc. are generally observed (7).

25(OH)D in serum is 25-hydroxyvitamin D. Vitamin D deficiency (50 nmol/L or 20 ng/ml) is commonly associated with fractures and bone loss, both of which are detrimental to skeletal health. The primary treatment goal is therefore a 25(OH)D concentration of >50 nmol/L or 20 ng/ml, although some data suggest a benefit at a higher threshold. 25(OH)D concentration of less than 30 nmol/L (or 12 ng/ml) indicates a deficiency in vitamin D, which substantially raises the risk of infections, mortality, and a number of other conditions; precautions should be taken to avoid this condition (8). Clearly, vitamin D is not a complete cure, but it is likely mostly beneficial in deficiency. Due to its uncommon side effects and relatively large margin of safety, it may be an effective, reasonable, and risk-free adjuvant treatment for a variety of disorders (9). The transport of proteins in the production of calcium in the small intestine, which is stimulated by vitamin D, is crucial for enhancing the absorption of dietary calcium. This diminishes the likelihood of osteomalacia in adults and rickets in children. When exposed to ultraviolet (UV) light from the sun, the body can produce adequate quantities of vitamin D. When exposure

to sunlight is limited, blood levels of 25-hydroxyvitamin D (25(OH)D) must be maintained through dietary sources of vitamin D (10).

Mushrooms are distinct organisms pertaining to the kingdom of fungi, the majority of which are edible despite the presence of highly toxic species. Mushroom cell walls contain high levels of ergosterol, which helps to reinforce cell membranes, modulate membrane fluidity, and facilitate intracellular transport (11). Oyster mushroom (*Pleurotus florida*), known as 'dhingri' in India, grows spontaneously on decaying logs or on fading trunks of deciduous or coniferous trees in temperate and tropical woodlands (12). *Pleurotus florida* is classified within the Class Basidiomycete and Agaricaceae family. The Latin and common names refer to the morphology of the fruiting body. The Latin word "Pleurotus" means sideways, which refers to the plant's sideways growth, while the Spanish term "florida" refers to a flower-like appearance. Worldwide, *Pleurotus florida* is now cultivated for human consumption. Mycoremediation is an industrial application for oyster mushrooms (13), (14). The cultivation of oyster mushrooms has grown in popularity due to their low production costs, simplicity of cultivation, and high nutritional value (15). The Samhitas of Ayurveda (16) and the Nighantu (17) both contain references to various varieties of mushrooms. In Bhavaprakasha (18), the poisonous, non-poisonous, and palatable varieties of Chatraka (Mushrooms) are described in detail. The gills of white oyster mushrooms cultivated in a sanitary environment are umbrella-shaped and contain high levels of nutrients (19).

Upon exposure to UV radiation such as UV rays or sunlight, commonly edible mushrooms generate higher concentrations of nutritionally significant Vit D. Mushrooms possess limited amounts of D3 and major amounts of D2 form of Vitamin D. Mushrooms' Vitamin D2 concentration may decrease with long storage and various cooking methods, but only if consumed within the "best-before" date and indicated cooking methods, the nutritive values are supplemented. Because the vitamin level remains above 10 g/100 g of fresh weight, which is a significant level compared to other Vitamin D-containing foods and meets the international daily requirement. Mushrooms are likely

the only non-fortified, non-animal source of vitamin D that contains a significant amount of vitamin D₂ per serving (11).

Pleurotus florida is cultivated in rice straw treated with Amalaki decoction as per the standard cultivating procedure. At the incubation period, 20-25°C temperature is optimized and the incubation room left undisturbed for 14 days. During fruiting, the Relative Humidity is maintained @70-85% by spraying Amalaki decoction. At least 8-10 hours of light/sunlight is provided during the time of fruiting. Harvesting performed from the fruiting body before spraying the decoction.

Aims and Objectives:

Aim: To study the efficacy of *Pleurotus florida* grown in Amalaki medium in Vitamin D deficiency.

Objectives:

Primary:

The assessment and comparison of the Vitamin D 25(OH) in blood before and after the study.

Secondary:

To evaluate and compare the variations in clinical symptoms like hair fall, fatigue, and muscle pains.

Materials and Methods:

A total number of 50 patients who fulfilled the inclusion criteria from Primary Health Centre Palakkad were screened and selected. They were treated with *Pleurotus florida* grown in Amalaki medium soup made of 100 grams of drug and administered thrice a week. Blood investigation Vitamin D 25(OH) is recorded before the commencement of treatment, 0th day, 30th day, 60th day, and 90th day during the course of treatment, and 30th day after the last dose is administered.

Mushroom Soup was made with 100gms of UV-treated *Pleurotus florida* grown in Amalaki medium which is cut into small pieces and 2gms of jeeraka churna, 2gms of maricha churna, and 1 gm of lavana cooked under pressure for five minutes with 4 times water. This soup is administered.

Inclusion/Exclusion Criteria

Inclusion Criteria:

Patients of age group in between 20 years and 50 years.

Patients of both genders.

Vegetarian diet.

Vit D deficiency mild to moderate (between 10-29ng/ml).

Exclusion Criteria:

Patients below the age of 20 and above the age of 50 years.

Transgenders excluded.

Patients with malignancy, TB, or any other systemic disorders.

Vit D deficiency which is severe (less than 10ng/ml).

Pregnant women.

Lactating mothers.

Non-vegetarian diet.

Study Type:

Single arm, Prospective, Interventional Clinical Study

Sample size:50 subjects

Assessment Criteria and Data Recording:

Compliance with the drug was recorded in four visits (30th day, 90th day, 60th day, and 30th day after completion of the administration) 30 days apart.

Vit D 25(OH) was performed during pre and post-clinical studies.

Assessment of clinical symptoms was performed during every visit.

A general physical assessment is recorded in Case Report Form throughout each visit.

Subjective Assessment:

Fatigue assessment questionnaire for the assessment of fatigue (21).

Patient standardized hair growth questionnaire used for assessment of hair loss (22).

Hair loss assessed through Hair pull test (23): A small section of hair around 20-30 stands, in sections from different parts

of the scalp are grasped and gently tugged or pulled. If >10% falls, it is considered active hair loss.

Grade of Fatigue in the complete study

1 = Never

2 = Sometimes [about monthly or less]

3 = Regularly [about few times a month]

5 = Often [about weekly]

6 = Always [about every day]

Grade of Hairloss in the complete study

1+ Minimal (Less than 5 strands pulled out)

2+ Minimum to moderate (5-10 strands pulled out)

3+ Moderate (11-15 strands pulled out)

4+ Severe (More than 16 strands pulled out)

Body pain assessed by Wong-Baker FACES Pain rating Scale

Statistical Analysis:

Classification of the Fatigue in the Study

Table1

Grade	Before administration of Pleurotus florida grown in Amalaki medium Soup		After the course of 03 months of Pleurotus florida grown in Amalaki medium Soup
	Frequency	Percentage	Nil fatigue was observed in all subjects
Nil	0	0	
Often	22	44	
Sometimes	1	2	
Regularly	5	10	
Always	22	44	
Total	50	100	

Classification of Hair fall in the Study

Table 2

Name	Hairloss	Before		After	
		Frequency	Percent	Frequency	Percent

Pleurotus florida grown in Amalaki medium	1+	0	0.0	49	98.0
	2+	0	0.0	1	2.0
	3+	45	90.0		
	4+	5	10.0		

Classification of Muscle pain in the Study

Table 3

Pleurotus florida grown in Amalaki medium	Pain Score	Before		After		Z-value (P- value)
		Freq	Percent	Freq	Percent	
	0	18	36	50	100	5.012** (<0.001)
	2.0	6	12			
	4.0	15	30			
	6.0	8	16			
	8.0	3	6			
	Mean $\pm$ SD	2.88 $\pm$ 2.59		0 $\pm$ 0		
Median $\pm$ (IQR)	4 (4)		0 (0)			

** Significant at 0.01 level

Vitamin D Mean Score and Standard Deviation of the group at different durations:

Vitamin D was assessed by blood investigation -Vitamin D 25(OH) at Vithayathil Laboratory, Palakkad. The blood was collected at PHC Malampuzha Palakkad by the Lab technician and was subjected to the investigation through cobas e 411 analyzer.

Comparison of vitamin D score of cases between days in each group was done separately by using Repeated measures ANOVA followed by Least significant difference (LSD) test.

Table 4

Day	Mean	Std Deviation
Day 0 (Initial Day)	11.33	1.10
Day 30	14.98	1.24
Day 60	24.30	2.02
Day 90	32.09	2.65
Day 121	32.09	2.65
P-value	< 0.001	

** Significant at 0.01 level

Results of pairwise comparison of vitamin D deficiency between days in

Pleurotus florida grown in Amalaki medium group

Table 5

Day (I)	Day (J)	Mean Difference (I-J)	Std. Error	P-value
Day 0	Day 30	-3.643**	0.152	<0.001
	Day 60	-12.963**	0.491	<0.001
	Day 90	-20.755**	0.569	<0.001
	Day 121	-20.753**	0.569	<0.001
Day 30	Day 60	-9.319**	0.414	<0.001
	Day 90	-17.111**	0.495	<0.001
	Day 121	-17.110**	0.494	<0.001
Day 60	Day 90	-7.792**	0.265	<0.001
	Day 121	-7.791**	0.265	<0.001
Day 90	Day 121	0.002*	0.004	0.031

** significant at 0.01 level; * significant at 0.05 level

Discussion:

Vitamin D is a growing public health concern. Vitamin D deficiency ranges from 40 to 99 percent prevalence and is one of the leading causes of ill health in the current context. Vitamin D deficiency is identified by measuring Vitamin D 25(OH) in the blood. Compared to osteomalacia and rickets, the symptoms of Vitamin D deficiency, such as fatigue, hair loss, fibromyalgia (muscle pain), and melancholy, are extremely prevalent in daily life. Vitamin D is essential for sustaining the optimal serum calcium level in the body. Reduced vitamin D levels result in decreased serum calcium concentrations. Low calcium levels cause hair loss, fatigue, and generalised body pain, among other symptoms.

Mushrooms are a timeless food that the majority of humans favour. Macrofungi have been consumed as sustenance since the beginning of time. Including mushrooms in one's diet on a regular basis contributes to a healthier lifestyle by supplementing the majority of essential nutrients. In addition to providing employment,

mushroom cultivation helps convert agricultural waste into beneficial products, thereby reducing environmental pollution. For humankind, mushrooms have expanded not only as a dietary source, but also as cosmeceuticals, nutraceuticals, and pharmaceuticals. Since raw mushrooms have a very limited shelf life, it would be prudent to investigate dry forms of the same without sacrificing quality in order to extend their shelf life. If combined with other plant products, *Pleurotus florida* could be an effective treatment for a number of diseases, including cardiovascular disease, stroke, autoimmune diseases, diabetes, etc.

When exposed to sunlight after harvesting, *Pleurotus florida*, also known as the white oyster mushroom, possesses a significant concentration of vitamin D. When used within the shelf-life period and with the recommended cooking methods, avoiding over-frying or prolonged exposure to heat, *Pleurotus florida* effectively combats vitamin D deficiency. The popularity of *Pleurotus florida* has increased because of its nutritional value, minimal production costs, and ease of cultivation. It flourishes between 28 and 30 degrees Celsius. Amalaki, the Indian gooseberry is also well known for its nutraceutical and pharmaceutical properties. It is a very rich source of Vitamin C and calcium. When used in cultivation of white oyster mushroom, probably it would enhance the therapeutic effect of the same.

Soup belongs to a therapeutic diet that is increasing its popularity now a days. Soup is a one of the apt diet in the unwell condition. It is the preparation with large portion of water and with the ingredients specific to the disease condition, making it easily available to the system for its digestion and absorption as the digestive capacity is impaired in the ill. Disease specific soup is advised which enhance the effect of treatment. As Soup is simple to prepare and easily digestible, it can be readily incorporated into a long-term diet. Soup made of *Pleurotus florida* is made with 100gms of drug and is administered thrice a week for a period of 03 months.

44% of subjects reported 'Always' fatigue, while 02% reported 'Sometimes' fatigue, 44% 'Often' fatigue, 10% 'Regularly' fatigue, and 0% 'Nil' fatigue before the trial began. After the conclusion of the experiment, no

participant reported fatigue. Before the clinical trial, the "Wong-Baker FACES Pain rating Scale" revealed that 36% of subjects had no muscle pain, 12% had grade 2, 30% had grade 4, 16% had grade 6, and 06% had grade 8 pain. After the clinical trial, all participants experienced absence of muscle pain. This may be a result of vitamin D supplementation, as well as its antioxidant and immunomodulatory properties and nutritional values, such as glycoproteins, polysaccharides, flavonoids, terpenoids, lectins, steroids, etc present in *Pleurotus florida* and the effect of amalaki used for the culture of white oyster mushrooms. Hair fall was observed 1+ in 0% of the subjects, 2+ in 0%, 3+ in 90% and 4+ in 10% of the subjects prior to the start of clinical trial. After the clinical trial, grade 1+ hair loss was observed in 98% of the subjects, and grade 2+ hair loss was observed in 2%. This could be the result of the optimum Vitamin D supplementation which in turn improves the calcium absorption and in turn reduces the hairfall.

Blood investigation of Vitamin D 25(OH) was performed before the commencement of the trial, on 30th day, 60th day, 90th day and 30 days after the completion of the 03 months. In this study, it has been observed that there is a significant increase in Vitamin D levels from Day 0 to Day 30, from Day 30 to Day 60 during the period of study, and after 30 days-after the last dose is administered, the vitamin D levels were appreciable. The P value was <0.001 and is significant at 0.01 level from Day 0 to Day 30, Day 60, Day 90 & Day 121. After the clinical trial period on Day 90, the Day 121 showed the P value 0.031 which is significant at 0.05 level. The Vitamin D content of UV-exposed *Pleurotus florida* grown in amalaki medium, when administered as readily digestible soup, would have supplemented the Vitamin D deficiency significantly.

Conclusion:

Mushrooms, because of their significant contribution to the treatment of numerous degenerative diseases, have long been prized and admired. *Pleurotus florida* can be grown in different substrates and is easily cultivated. On exposure to UV radiations, the vitamin D content increases in *Pleurotus florida*. Soup is easily digestible and can be prepared with ease. Supplementing Vitamin D with

naturally occurring products may help in better outcome than the chemically generated supplements.

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