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Clinical Evaluation of *Pleurotus florida* (White Oyster Mushroom) – *Chatraka* in the management of Vitamin D deficiency

Research Article

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Abstract

Oyster mushroom (*Pleurotus florida*) commonly called in India as '*dhingri*', develops spontaneously on dead and rotting wooden logs or sporadically on dying trunks of deciduous or coniferous trees in temperate and tropical woodlands. *Pleurotus* cultivation not only helps in supplementing the nutritional needs, but also offers an opportunity for self-employment along with mycoremediation. *Pleurotus florida* is cultivated in the paddy straw following the standard cultivation procedure. After culture, on exposure to sunlight or UV lamp, oyster mushrooms produce concentrations of Vitamin D that are nutritionally significant. Though long periods of storage and different types of cooking tend to decrease the vitamin D level of UV-exposed oyster mushrooms, the vitamin D level in all probability expected to remain at 10ug/100g fresh weight, which is greater than the amount in the majority of foods containing vitamin D 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 *asthikshaya*. *Kesa patina, shareera shoola* and *srama* 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: *Pleurotus florida*, UV lamp, Vitamin D, Soup, Mushrooms.

Introduction

Vitamin D generally called the sunshine vitamin, is fat-soluble with well-known antirachitic properties (1). Calciferols refer to a group of fat-soluble seco-sterols, both cholecalciferol which is Vitamin D3, and ergocalciferol which is Vitamin D2 both of which are lipid-soluble compounds with a 4-ringed cholesterol backbone (2). Vitamin D refers to Vitamin D3 in general. Vitamin D can be produced naturally. Approximately 90% of the necessary D Vitamin is synthesized by the epidermis as a result of sun exposure (3). Inadequate vitamin D status is becoming a worldwide epidemic (4). Prevalence of vitamin D deficiency ranges from 40% to 99%, with the bulk of research claiming a prevalence of 80%–90% (5). An estimated one billion individuals worldwide, of all races and ages, are deficient in D Vitamin. An independent risk factor for overall mortality in the general population is hypovitaminosis D making vitamin D deficiency a particularly significant public health concern. A health intervention worldwide supplementation of D Vitamin to prevent its extreme deficit by dietary fortification would appear to be crucial (6). Vitamin D deficiency symptoms are mostly asymptomatic, but complaints of fatigue, insomnia, depression, hairloss, muscle weakness and pain, loss of appetite etc. (7).

Serum 25-hydroxyvitamin D [25(OH)D] Vitamin D deficiency (<50 nmol/L or 20 ng/ml) is generally linked to fractures and bone loss which are unfavorable to skeletal outcomes. The primary treatment objective is, therefore, a level of >50 nmol/L or 20 ng/ml of 25(OH)D, although some data imply benefit in a higher threshold25(OH)D concentration of less than 30 nmol/L (or 12 ng/ml) indicates a lack of vitamin D which significantly increases the risk of infections, mortality, numerous other disorders, and care ought to be taken to avoid this situation (8). Clearly, not a miracle cure, vitamin D is likely only beneficial in deficit. Providing its uncommon consequences and comparatively large safety margin, it may be an effective, reasonable, and risk-free adjuvant treatment for a variety of disorders (9). For improving the absorption of dietary calcium, in the small intestine's production of calcium, the transport of proteinsis essential which in turn gets stimulated by D Vitamin. This reduces the probabilities of osteomalacia and rickets in adults and children respectively. The body can 9produce vitamin D in adequate quantities when subjected to the sun's

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ultraviolet (UV) light. When sunlight exposure is limited, the levels of 25-hydroxyvitamin D (25(OH)D) in the blood must be maintained through dietary sources of vitamin D (10).

Mushrooms are distinct biological entities belonging to the fungi kingdom and most of them are edible although highly poisonous varieties are not to be ignored. Mushroom cell walls possess high concentrations of ergosterol, which help strengthen cell membranes, regulate membrane fluidity, and facilitate intracellular transport (11).

Oyster mushroom (*Pleurotus florida*) commonly called in India as '*dhingri*', develops spontaneously on dead and rotting wooden logs or sporadically on dying trunks of deciduous or coniferous trees in temperate and tropical woodlands (12). *Pleurotus florida* belongs to the Class Basidiomycetes and Family Agaricaceae. The Latin and common name refer to its fruiting body morphology. The Latin *Pleurotus* means sideways which refers to the sideways growth, whereas the Spanish word *florida* means a feast of flowers for its flower-like appearance. *Pleurotus florida* is now cultivated worldwide for human consumption. Oyster mushrooms can be used industrially for mycoremediation(13),(14). Oyster mushroom cultivation has gained popularity because of its low production cost, ease of cultivation, and high nutritional value (15). References to different types of mushrooms can be found in the *Samhita* (16) period as well as elaborated descriptions in the *Nighantu* (17) period. A detailed explanation of the poisonous and non-poisonous and edible varieties of *Chatraka* is described in *Bhavaprakasha* (18). White oyster mushroom which is grown in a hygienic environment is white in colour and the gills are umbrella shaped and possess high nutritive values. *Pleurotus florida* should be considered as *Chatraka* than the button mushroom as it is white in colour with umbrella like cap and possess high nutritive value (19).

On exposure to UV radiation such as UV lamps or sunlight, commonly consumed mushrooms produce higher concentrations of nutritionally significant Vit D. Mushrooms possess the D2 form of Vitamin D with limited amounts of D3. With long storage and different types of cooking, the Vitamin D2 concentration of mushrooms may decrease, but if consumed within the "best-before" period. Because Vitamin level remains above 10 µg/100 g fresh weight which is a significant level compared to other foods which contain Vitamin D and commensurate to the international daily requirement. Mushrooms probably are the only non-fortified, non-animal Vitamin D source that provides a substantial quantity of vitamin D2 per serving (11).

Aims and Objectives

Aim: To study the efficacy of *Chatraka* soup 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 (*Keshapata*), fatigue (*daurbalya*), and body pains (*shareera shoola*).

Materials and Methods

A total number of 50 patients who fulfilled the inclusion criteria from Primary Health Centre Malampuzha were screened and selected. They were treated with *Pleurotus florida* (*Chatraka*)soup made of 100 grams of *chatraka* 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.

Chatraka Soup was made with 100gms of UV-treated *samoola chatraka* 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 (150ml of soup thrice a week is once a day) is administered.

- *Rasa Panchaka* of *Chatraka* (20):
- *Rasa: Madhura*
- *Guna: Guru*
- *Vipaka: Madhura*
- *Veerya: Sheeta*
- *Karma:Vrushya, Balya*

According to the article – "koonite rajyam", written by Dr KBG Nayar, the white oyster mushroom – *Pleurotus florida* – is considered as Chatraka on the basis of the explanation of *Bhavaprakasha*. *Bhavaprakasha* explains that *samswedaja saka* grown in clean environment, white in colour and resembles umbrella is to be taken. *Pleurotus florida* falls better in this category in comparison with button mushroom (*Agaricus campestris*) and hence can be considered as *Chatraka. Chatraka* is a synonym of *Samswedajasaka.*

Inclusion/Exclusion Criteria

Inclusion Criteria

- Patients of age group in between 20 years and 50 years.
- Patients of both genders.
- Vegetarian diet. [Few fishes like salmon are good source of Vitamin D. Hence excluded]
- 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. (Few fishes like salmon and meat are good source of Vitamin D. Hence excluded)

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).

The FAS is a 10-item scale evaluating symptoms of fatigue. Michielsen and colleagues developed and analysed the scale's psychometric properties and found an internal consistency of 90.

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 [This gradation was based on fatigue questionnaire itself]

- 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 [through Hair pull test-to reconfirm the hair loss] - Measured by the number of hairs fallen in each pulling.

- 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)

Patients approaching the clinic with fatigue and hairloss were screened for Vitamin D deficiency through the blood investigation – Vitamin D 25(OH). As the study was conducted in the Public Health Centre, approached patients were not aware of the Vitamin D deficiency. Vitamin D deficiency leads to *asthi kshaya* which in turn leads to *kesapatana, shareera shoola* and *srama*. Hence these parameters were taken.

Body pain assessed by Wong-Baker FACES Pain rating Scale (24)

The Wong-Baker Faces Pain Rating Scale is a method for someone to self-assess and effectively communicate the severity of pain they may be experiencing. The scale contains a series of six faces ranging from a happy face at 0 to indicate "no hurt" to a crying face at 10 to indicate "hurts worst."

Statistical Analysis

Table 1: Classification of the Fatigue in the Study

Grade	Before administration of *Pleurotus florida* Soup		After the course of 03 months of *Pleurotus florida* Soup
	Frequency	Percentage	
Nil	7	14	Nil fatigue was observed in all subjects
Often	11	22	
Sometimes	13	26	
Regularly	3	6	
Always	16	32	
Total	50	100	

Table 2: Classification of Hair fall in the Study

Name	Hairloss	Before		After	
		Frequency	Percent	Frequency	Percent
Pleurotus florida	1	4	8.0	41	82.0
	2	3	6.0	9	18.0
	3	37	74.0		
	4	6	12.0		

Table 3: Classification of Body pain in the Study

	Pain Score	Before		After		Z-value (P-value)
		Freq	Percent	Freq	Percent	
Pleurotus florida	0	20	40	50	100	4.832** (<0.001)
	2.0	2	4			
	4.0	8	16			
	6.0	10	20			
	8.0	10	20			
	Mean ± SD	3.54 ± 3.25		0 ± 0		
	Median (IQR)	4 (6)		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)	11.795	1.384
Day 30	15.343	1.844
Day 60	22.576	3.957
Day 90	29.544	4.322
Day 121	29.533	4.318
P-value	< 0.001	

Table 5: Results of pairwise comparison of vitamin D deficiency between days in Pleurotus florida group

Day (I)	Day (J)	Mean Difference (I-J)	Std. Error	P-value
Day 0	Day 30	-3.548**	0.152	<0.001
	Day 60	-10.781**	0.491	<0.001
	Day 90	-17.749**	0.569	<0.001
	Day 121	-17.738**	0.569	<0.001
Day 30	Day 60	-7.234**	0.414	<0.001
	Day 90	-14.201**	0.495	<0.001
	Day 121	-14.190**	0.494	<0.001
Day 60	Day 90	-6.967**	0.265	<0.001
	Day 121	-6.956**	0.265	<0.001
Day 90	Day 121	0.011*	0.004	0.031

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

Discussion

Vitamin D is an increasing concern in public health. The prevalence of vitamin D deficiency ranges from 40% to 99% and is one of the main causes of ill health in the present scenario. Vitamin D deficiency is identified by the blood investigation of Vitamin D 25(OH). The symptoms of Vitamin D deficiency like fatigue, hair loss, fibromyalgia (muscle pain), depression are very common in day to day life compared osteomalacia and rickets. Vitamin D plays a crucial role in maintaining the optimum level of serum calcium in the body. Reduced vitamin D leads to reduction in serum calcium levels. Reduced calcium levels lead to *asthi kshaya*. The symptoms of asthi kshaya includes *kesha patana* and *srama*.

Mushrooms are an all-time cuisine preferred by the majority of human beings. Macro fungi have been used as food from time immemorial. The use of mushrooms regularly as a diet helps in a better healthy living as it supplements most of the essential nutrients. Mushroom cultivation not only provides employment but also helps to convert wastes from agriculture into useful produce thus reducing environmental pollution. Mushrooms have expanded up to a wider extent not only as food but also in the area of cosmeceuticals, nutraceuticals, and pharmaceuticals for mankind. Mushrooms in their raw state have a very short shelf life; therefore, 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 variety of diseases, such as heart disease, stroke, autoimmune diseases, diabetes, etc.

Pleurotus florida commonly known as white oyster mushroom when exposed to sunlight after harvesting possesses a significant concentration of Vitamin D when used within the shelf-life period and with indicated cooking methods avoiding over-frying or exposure to heat for a longer period, *Pleurotus florida* (*Chatraka*) helps in combating the vitamin D deficiency effectively. *Pleurotus florida* has gained popularity due to its nutritional value, low-production cost, and ease of cultivation. It grows at 28-30 degrees temperature. According to Ayurveda *Chatraka* which is *shweta* and that which is grown in a hygienic place is edible and is *vrushya* and *balya* with *Madhura rasa*, *guru guna*, and *sheeta veerya*.

Yusha belongs to a therapeutic diet that is increasing its popularity now a days. *Yusha* 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 *Agni* (digestive capacity) is impaired in the ill. Disease specific *yushas* are suggested which makes the treatment much more effective. As *yusha* is simple to prepare, easily digestible, and can be readily incorporated into a long-term diet. It is said that *Pathya kalpana* prevents the progression of the disease if consumed in *Sanchayavastha* (prior to the onset of a disease), and in *Kupitavastha* (during the progressive stage). *Yusha* made of *Pleurotus florida* is made with 100gms of drug and is administered thrice a week for a period of 03 months.

32% of subjects reported 'Always' fatigue, while 26% reported 'Sometimes' fatigue, 22% 'Often' fatigue, 6% 'Regularly' fatigue, and 14% '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 40% of subjects had no muscle pain, 4% had grade 2, 16% had grade 4, 20% had grade 6, and 20% 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*. Hair fall was observed 1+ in 8% of the subjects, 2+ in 6%, 3+ in 74% and 4+ in 12% of the subjects prior to the start of clinical trial. After the clinical trial, grade 1+ hair loss was observed in 82% of the subjects, and grade 2+ hair loss was observed in 18%. This could be the result of the optimum Vitamin D supplementation which in turn improves the calcium absorption and in turn reduces the *asthi kshaya lakshanas*.

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 0.01level 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 0.05level. The Vitamin D content of UV-exposed *Pleurotus florida*, when administered as readily digestible *yusha*, 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*. *Yusha* 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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