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Inhibition of Germ Tube Formation in Mucor & Candida by JRK's DCOD

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ABSTRACT

JRK's DOCD is a proprietary siddha medicine, indicated for prevention of co-morbidities of diabetes. Earlier studies on the product has proven its effect in boosting the phagocyte mediated immunity. In the current study the ability of the product in preventing pathogenicity of fungi is evaluated. Germ tube formation is proven to increase the pathogenicity of the fungi than the spores alone. For this different species of fungi i.e., *Candida albicans*, 2 isolates of *Candida dubelensis* and three isolates of Mucor and 1 isolate of Rhizopus are taken. These fungal cultures when treated with the JRK's DCOD, there is an inhibition of germ tube formation compared to control. Complete study details are presented in the paper.

KEYWORDS: JRK's DCOD, Mucormycosis, Candida, Diabetes Mellitus Treatment, Germ Tube Formation

INTRODUCTION

Most of the fungi that are reported to infect man are largely opportunistic pathogens and lack true virulence potential (1). Fungi in general are heterotropic or saprophytic organism and always look for such habitat to colonize and procreate. The most common fungal infections encountered among diabetic population are candida infection and mucormycosis (2). Candida is yeast like fungi belonging to the class basidiomycetes whereas mucor belongs to the class zygosporale.

For the initiation of these opportunistic saprophytic/ commensal fungi to become pathogenic, severe immune compromised state of the host is needed (4). Diabetic population possibly due to impaired phagocyte mediated immune defense and also due to hyperglycemia are highly susceptible to fungal invasion.

DCOD is a poly-herbal formulation with proven scientific evidence in regulating glucose metabolism by the cells of various organs in our body at in-vitro level (5-9). Further the DCOD also increases the sensitivity of cells towards insulin and give a body blow to various breakdown cellular products such as creatine, creatinine etc., by eliminating them from blood stream and thereby reduces the renal burden.

In a separate study, we have also established some of the herbs in DCOD boost the phagocyte mediated immunity against wide spectrum of antigens such as carbon particles, opsonized and or non-opsonized spore preparations of Aspergillus, bacteria etc.

The secretory myeloperoxidase as a result of increased phagocytosis was also suppressed by DCOD at laboratory level. The above cascade benefits of DCOD is being duly recognized by several Diabetologists and are prescribing DCOD tablet dosage form to patients starting from pre-diabetic to border line diabetes mellitus to the level where co-morbidity has progressed, for prevention, protection and possibly for the restoration of the organ health. DCOD is also gaining significant acceptance among people who have definite diabetic family history.

The mycological events that prelude mucor and candida invasion starts with the spore ingestion, angio-invasion, hemotogenic distribution, visceral navigation and finally the tissue colonization (10).

Although the spores of the above fungi are heavily loaded in the air but even the susceptible individuals also will not develop infection so easily due to the weaker ability of the spores to initiate most of the mycological events. Further the monocytes, phagocytes in the blood stream, alveoli and in the peritoneal cavity destroy the spore as soon as they enter the system.

Recent studies have proved that the spores with germ tube are the one which has greater invasion ability and potentially initiates all the cascade events of pathogenesis. However, the spores as well as spores with germ tubes have equal cell adhesion ability.

In the present study we have established the effect of DCOD in inhibiting germ tube formation in mucor spores as well as

in candida cells in three different conditions simulated to mimic near human host.

The additional germ tube inhibition effect of DCOD is likely to offer the much needed protection to diabetic people from fungal invasion due to the most common opportunistic fungal pathogens. Details are presented in the paper.

MATERIALS AND METHODS

Serum and serum supplemented Brain heart infusion broth, RPMI 1640, Dulbacos modified Eagles medium, Sabauraud's dextrose broth were used for the present study.

Three isolates of Candida albicans, 2 isolates of Candida dubelensis and three isolates of Mucor and 1 isolate of Rhizopus were used for the present study.

Freshly grown Candida cells were prepared as suspension in normal saline and the cells were adjusted to 100 cells per milliliter of the saline with the help of Haemocytometer. Similarly the spore suspension was prepared for other fungi as well by employing the same method.

INOCULATION PROCEDURE

The adjusted fungal cell suspension and spore suspension were inoculated to 1 ml of serum, brain heart infusion broth, RPMI 1640, Dulbacos modified eagle's broth and Sabaurauds dextrose broth separately and incubated for 3 hrs. After 30 minutes, 1, 2 and 3hrs of incubation, the suspension was observed under microscope for germ tube formation. The % of cells produced germ tube was calculated. The above cell and spore suspension in different culture conditions in triplicate was maintained with different concentrations of DCOD extract conglomerate such as 0.2, 05 and 0.7 µg/ml and after incubation at the pre-determined time points, microscopic examination was done to understand the germination event.

Re-inoculation of fungal spores onto SDA

The fungal spores/cells treated both in DCOD and control set were plated onto the SDA and incubated for three days to check the rate of fungal growth.

Spore revival versus treatment time

The fungal spores and cells were collected aseptically in a pre-sterilized paper bag and the spores were stored at desiccated condition for 30 days and then the spores were inoculated onto SDA to assess the revival process.

RESULTS

Incorporation of DCOD despite concentration significantly inhibited germ tube formation in Candida albicans. In serum where 99% of cells produced germ tube in 1-3 hrs but in the same condition when DCOD was incorporated, the germ tube formation was observed only in20-30% of cells. Table 1. The germ tube formation inhibition was consistent in all different culture media used in the present study.

Table 1. Germ tube formation in three isolates of *Candida albicans* (values presented from average)

Culture condition	Germ tube / Percentage			
	30min	1 hr	2 hr	3hr
Serum (untreated)	90	99	99	99
RPMI 1640 (untreated)	60	60	70	70
DMEM (untreated)	50	50	50	60
SD (untreated)	5	10	10	10
Serum + 0.2 DCOD	20	30	30	30
Serum + 0.5 DCOD	10	15	20	20
RPMI1640 + 0.2 DCOD	5	10	10	10
RPMI 1640 + 0.5 DCOD	5	5	5	5
DMEM +0.2 DCOD	10	10	10	10
DMEM +0.5 DCOD	5	5	10	10
SD + 0.2 DCOD	5	5	5	5
SD + 0.5 DCOD	5	10	10	10

In Candida dubelensis, the germ tube formation remarkably got arrested in serum when DCOD was incorporated and reduction was 100% in RMPMI 1640 medium suggesting the strong germ tube formation inhibition effect of DCOD Table 2.

Table 2. Germ tube formation in three isolates of *Candida dubelensis*

Culture condition	Germ tube / Percentage			
	30min	1 hr	2 hr	3hr
Serum (untreated)	100	100	100	100
RPMI 1640 (untreated)	70	80	100	100
DMEM (untreated)	20	30	30	40
SD (untreated)	-	10	20	20

Serum + 0.2 DCOD	10	10	20	20
Serum + 0.5 DCOD	5	10	10	10
RPMI1640 + 0.2 DCOD	5	5	5	5
RPMI 1640 + 0.5 DCOD	-	-	5	5
DMEM +0.2 DCOD	5	5	5	5
DMEM +0.5 DCOD	-	-	-	5
SD + 0.2 DCOD	-	-	-	-
SD + 0.5 DCOD	-	-	-	5

Compared to the species of Candida, Mucor and Rhizopus did not produce extensive germ tube formation in serum or other media used however, DCOD has shown strong effect of germ tube formation inhibition in Mucor and Rhizopus also Table 3.

Table 3. Germ tube formation in the isolates of Rhizopus and *Mucor spp.* (values presented from average)

Culture condition	Germ tube / Percentage			
	30min	1 hr	2 hr	3hr
Serum (untreated)	40	60	60	60
RPMI 1640 (untreated)	50	50	50	50
DMEM (untreated)	40	40	40	40
SD (untreated)	-	-	-	-
Serum + 0.2 DCOD	10	20	20	20
Serum + 0.5 DCOD	10	10	10	10
RPMI1640 + 0.2 DCOD	10	10	10	10
RPMI 1640 + 0.5 DCOD	5	5	10	10
DMEM +0.2 DCOD	5	5	5	5
DMEM +0.5 DCOD	-	-	5	5
SD + 0.2 DCOD	-	-	-	-
SD + 0.5 DCOD	-	-	-	-

Significant reduction in the rate of revival of fungal spores of all fungi tested was observed in the condition where DCOD treatment was employed whereas complete recovery was observed in DCOD untreated conditions Table 4.

Table 4. Revival rate of fungal spores post treatment with DCOD

Culture condition	% Rate of revival in SDA			
	Day 3	Day 5	Day 7	Day 10
Serum (untreated)	100	100	100	100
RPMI 1640 (untreated)	100	100	100	100
DMEM (untreated)	100	100	100	100
SD (untreated)	100	100	100	100
Serum + 0.2 DCOD	40	40	40	40
Serum + 0.5 DCOD	40	40	40	40
RPMI1640 + 0.2 DCOD	40	40	40	40
RPMI 1640 + 0.5 DCOD	40	40	40	40
DMEM +0.2 DCOD	40	40	40	40
DMEM +0.5 DCOD	40	40	40	40
SD + 0.2 DCOD	40	40	40	40
SD + 0.5 DCOD	40	40	40	40

Spore revival versus treatment time

Spores of mucor, rhizopus and cells of candida species could grow and become a colony in SDA after 30 days of desiccated storage conditions.

DISCUSSION

The germ tube formation experiment by the spores of different species of fungi such as Candida albicans, Candida dubelensis, Mucor spp., Rhizopus spp., has revealed that the herbal concoction or conglomerate can potentially inhibit the germ tube formation.

Whether such inhibition is the outcome of spore coating of the phytoactives or the selective destruction of the newly formed germ tube or through some unknown mechanism where the nutrients transport into the cell is being hindered is not clear. However our subculture experiment has shown that the spores that could not produce germ tube in DCOD plus serum supplemented condition were viable and active to form new colonies when they were transferred to new culture condition where the herbal depletion was quite obvious and pronounced.

Therefore we assume that the herbal constituents in DCOD may be inhibiting germ tube formation selectively and may not be damaging the spores. The emasculation of germ tube formation by the fungal spores assumes high medical importance in the scenario where many opportunistic fungal pathogens are gaining advantage to infect the predisposed hosts; obviously the disease diabetic mellitus ranks the first among disorder to sends out a red-carpet welcome to fungal invasion (13).

The growth inhibition assay also proved that the herbal constituents in DCOD do not have any potential antifungal effect. Another set of experiment to understand how long the spores would be viable under quiescent state in DCOD supplemented condition vis-à-vis a condition devoid of DCOD clearly proves that prolonged germ tube suppression despite nutrient support actually inactivate and or immobilize the spores and therefore after 5 days the spores could not grow when they were transferred to fresh set of culture condition. Whereas the spores stored in desiccated condition (the spores were in quiescent state) devoid of DCOD treatment revived back when they were seeded onto culture media even after 30 days. The above findings clearly suggest that DCOD through an unknown mechanism may be selectively targeting the germ tube formation in the tested group of fungi which have great medical importance in diabetic condition.

Germ tube formation in fungi when they are grown in serum supplemented media is a sign of potential virulence because the mycological events in the pathogenesis start with angioinvasion, hematogenic distribiton, visceral navigation and then colonization. The spores with germ tube prior to angioinvasion are reported to have potential virulence than just spores devoid of germ tube and that may be the reason why the spores or cells of these fungi in general do not cause infection in vast majority of the population. Therefore inhibiting the germ tube formation also needs to be considered as an important treatment strategy of fungal infection among diabetic and other vulnerable population.

The question is how such germ tube inhibition effect of DCOD would help the patients once infection is initiated. The question can be easily answered and we must make clear distinctions between the treatment which is oriented towards possible cure and the treatment strategy which is largely focused on prevention.

The preventive strategy is the key hallmark in medical science especially when we know in advance and have clearly identified the vulnerable population. When certain population is vulnerable, instead of pushing them through the drudgery of treatment post infection, we must muster on preventive measures so that the scope of infection, treatment and finally treatment success or failure, all can be avoided.

In our other set of experiments where we have loaded the fungal spores over egg semi-permeable membrane pre-coated with DCOD has shown that the spore adhesion was significantly low when DCOD treatment was applied over the membrane than the control.

Whether the cellular adhesion of the spores is being selectively inhibited by DCOD or as a consequence of germ tube formation inhibition has occurred, warrants further investigation. The mechanism involved in the vegetative growth of any fungi in general is different from germ tube formation from spores and hence the selective inhibition of germ tube formation has high medical significance.

DCOD in tablet dosage form is an accepted line of treatment for diabetes mellitus where the drug is proved at the laboratory level to increase the cellular glucose metabolism by the cells of several organs and also it cleaves the cellular breakdown bi-products such as creatine, creatinine etc. Further the inflammatory modulator-meyloperoxidase enzyme due to hyper phagocytosis is also been scissored by DCOD.

DCOD is being recommended to diabetic population along with the other main line treatment, regularly. Therefore the additional benefit that we have established recently about the germ tube formation inhibition effect of DCOD gives one more armor to diabetic population to combat the possible opportunistic fungal pathogens at the preventive level.

In our earlier study we have also established the effect of certain herbs in DCOD can increase the phagocyte mediated immunity against carbon particles as well as opsonized and non-opsonised bacterial and fungal spores at the laboratory level. The combination of benefits offered by DCOD essentially makes the herbal preparation indispensible in the treatment armamentarium of diabetes mellitus.

Since the diabetic population has been already red tagged as the most vulnerable to most diseases be it of infective origin or disorder linked, prevention and preventive medical strategy alone may decrease the disease burden in such population.

By protecting such vulnerable group in advance, we can, not only save millions of lives, also offer better quality of life to such population; we can also significantly decrease the disease burden and the subsequent spread of such diseases to others in the population. It also may boost the medical economy both at individual level and also to the nation.

DCOD is further formulated with extremely safe herbs which are being used vastly by general population even in some culinary preparations. Therefore DCOD comes with not only efficacy but also with absolute safety.

REFERENCES

1. Pfaller MA, Diekema DJ. 2004, Rare and Emerging Opportunistic Fungal Pathogens: Concern for Resistance beyond Candida albicans and Aspergillus fumigatus, J Clin Microbiol. 42(10): 4419–4431.
2. Syeda Neelam Afroze, Rajani Korlepara, Guttikonda Venkateswara Rao, Jayakiran Madala. 2017, Mucormycosis in a Diabetic Patient: A Case Report with an Insight into Its Pathophysiology, Contemp Clin Dent. 8(4): 662–666
3. Chian-Yong Low, Coleman Rotstein, 2011, Emerging fungal infections in immunocompromised patients, F1000 Med Rep. 2011; 3: 14. Published online 2011 Jul 1. doi: 10.3410/M3-14, PMCID: PMC3155160
4. Suzanne E. Geerlings, Andy I.M. Hoepelman, Immune dysfunction in patients with diabetes mellitus (DM), FEMS Immunology & Medical Microbiology, Volume 26, Issue 3-4, December 1999, Pages 259–265, https://doi.org/10.1111/j.1574-695X.1999.tb01397.x
5. Amruthavalli G.V., Aruna.V, Gayathri Rajagopal. Therapeutic Polymorphism' of anti-diabetic herbal drug. EC Diabetes and metabolic research, Vol 5, Issue 3, 2021.
6. Aruna.V, Amruthavalli G.V., Gayathri Rajagopal. Why poly herbal preparation is more advantageous than targeted single molecule based drug (s) for the management of diabetes mellitus. World Journal of Pharmaceutical and Medical Research. Vol 7, Issue 1, 2021.
7. Aruna.V, Amruthavalli G.V., Gayathri Rajagopal. Cellular Level Catalysation of Glucose by Organ Level Cells as a Possible Therapeutic Scope for Diabetes Mellitus. International Journal of Science and Healthcare Research.Vol.6; Issue: 1; Jan.-March 2021
8. Soundharya.R., Aruna.V, Amruthavalli G.V., Gayathri Rajagopal. Comparison on the Anti-Diabetic Effect of NIKU Plus Versus *Madhumega chooranam.* Research & Reviews: A Journal of Unani, Siddha and Homeopathy,Volume 6, Issue 2.
9. Amruthavalli G.V., Aruna.V, Gayathri Rajagopal. Solution for Diabetes mellitus-Niku plus, Journal of Diabetes and Treatment, Volume 2019, Issue 02.
10. Donald C. Sheppard, Scott G. Filler. 2015, Host Cell Invasion by Medically Important Fungi, Cold Spring Harb Perspect Med. 2015 Jan; 5(1): a019687. doi: 10.1101/cshperspect.a019687, PMCID: PMC4292075
11. Marianne Kretschmar, Bernhard Hube, Thomas Bertsch, Dominique Sanglard, Renate Merker, Meike Schröder, Herbert Hof, Thomas Nichterlein. 1999, Germ Tubes and Proteinase Activity Contribute to Virulence of Candida albicans in Murine Peritonitis, Infect Immun. 1999 Dec; 67(12): 6637–6642. PMCID: PMC97077
12. Ranjit Unnikrishnan, Anoop Misra. 2020, Infections and diabetes: Risks and mitigation with reference to India, Diabetes Metab Syndr. 2020 November-December; 14(6): 1889–1894. Published online 2020 Sep 22. doi: 10.1016/j.dsx.2020.09.022, PMCID: PMC7505871