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IN-VITRO INHIBITION OF GROWTH AND BIOFILM FORMATION OF CANDIDA ALBICANS BY NEEM LEAF EXTRACT

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Abstract
Candida albicans is a smart pathogen causing many human infections, and is notorious for biofilm fomation that makes treatment very difficult. Biofilm formation implies refractoriness to therapy. So herbal compunds are the need of the hour to tackle this proble. In this study we show by incubation with peptone water with neem extractya nd test tube mthod of biofilm study, that neem laves inhibited groth and biofilm in vitro by Candida albicans. Hence this extract should serve as source of future treatment options in infections caused by C. albicans.

INTRODUCTION

Neem's, (scientific name Azadirachta indica), is a traditional medicinal tree native to the Indian subcontinent. Various parts of the Neem tree and products derived from them have been traditionally used in India for their medicinal properties. Research over the past sixty years have studied the active compounds in Neem and validated their biological activity to explain the medicinal effects, leading to worldwide interest in this tree. Neem is an integral part of the Ayurvedic system of medicine, and medicinal use have been described for the leaves, roots, fruits, seed oil, and bark. The products from Neem tree have been demonstrated to exhibit immunity enhancing, anti-inflammatory, antidiabetic, antimalarial, antifungal, antibacterial, antiviral, and ant carcinogenic properties.[(1-2)]

Candida albicans is a yeast like fungus that normally is present on the human body, but its overgrowth leads to infection in different parts of the body like the genitourinary tract, skin, gastrointestinal tract, lungs and mouth. Extensive research studies have shown that extracts of neem leaf, neem oil and seed kernels are effective against Candida albicans.[(3)]

Neem leaves previously reported to have antibacterial properties and could be used to control airborne contamination[(4)]. This supported by the use of neem seeds in traditional medicine to treat infections[(5-7)]. Recently, in vitro study has demonstrated that extract of neem leaves prevent biofilm formation and adhesion in composite resin by C. albicans[(8)]. Moreover, the methanolic extract of neem was reported to have in vitro antimicrobial activities against S. aureus, E. coli, Ps. aeruginosa and C. albicans.[(9)]

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The purpose of the present study was to investigate anti virulent (effect on biofilm production, decrease number of colony count, effect on lipase activity and activity on cell wall) activity of neem plant leaves against Candida albicans.

MATERIALS & METHODS

This was a laboratory based observational study, carried out in the Department of Microbiology in the institute as a institutional (departmental) project, from January 2015 to November 2015. Clearance of institute ethics commitee was sought and obtained prior to the study. The test was performed for 6 randomly selected, clinical isolates of *Candida albicans*. Neem leaves were obtained from trees and plants in the hospital and residential complex, every time from different sources. Tests were done with mature leaves as well as young leaves.

Leaf Extracts Preparation: Fresh neem leaves were collected and the leaf was washed, dried, and chopped. Afterwards 8 grams of dried leaf were taken in a separate container, then 100 ml of peptone water was added to it, and autoclaved it at 121^0c at 15 lps/in^2 for 15 mins. Two (2) ml of peptopne water and peptone water with neem extract in sterile test tubes were prepare as two sets.

Method

The anti virulent activity of neem extract was evaluated against *candida albicans*. Six clinical isolates of candida albicans were randomly selected for the study.Isolated colonies (1 loopful) of *Candida albicans* were inoculated in both neem broth and peptone broth. Inoculated both broth were incubated overnight at 37^0C. After overnight incubation, *1 llopful of suspension*from both broth were again inoculated in SDA plate for colony count and on EYA(egg yolk agar) for checking lecithinase,lipase and protease activity assessment and incubated at 37^0c. Next day, colonies were observed for colony count reduction, and presence and alteration of lecithinase,lipase and protease activities. Lipase activity was detected by appearance of pearly shine on surface of colonies on Egg yolk agar. *Candida albicans* inoculated broths were gram stained to see the damage of fungal cell wall by indentifying transition from Gram's positive cells to Gram's negative cells. Antivirulence activity is also tested by comparing properties of biofilm formed from both broths. Biofilm formation was done by test tube method. After overnight incubation like above, supernatant broth from both tubes were discarded and washed thrice with normal sline; 0.5% safranine was added to both tubes and kept for 1 minute. After discarding safranine, tubes were again washed thrice with normal sline and kept in inverted position for visual observation of stained biofilms after drying. . Once biofilm formed,their properties were compared with each other. Reduction of colony count was calculated by Z-test of statistical significance. All tests were carried out thrice with each clinical strain.

For checking toxicity, 1 drop of the neem extract prepared, was mixed with 1 drop of buffy coat from blood samples kept in lab after processing (for tother tests), and mounting and observig microscopically for RBC/WBC cell lysis. This was also done thrice.

RESULTS

In current study, total six samples of *candida albicans* were isolated. Out of these 6 samples, 4 samples (66%) from neem broth showed decreased colony count, decreased lipase activity seen in one sample (16%),decrease biofilm adherence demonstrated in 4 sample (66%) and damage to cell wall by Gram's stain was seen in one sample (16%). Decreased colony count was calculated by Z-test of statistical significance[10]. Thus neem leaf extract inhibited growth (colony count) and biofilm method by *Candida albicans* in vitro. Effect was more marked with young leaves. It was aldso non-toxic to WBC and RBC since it did not cause cell lysis.

DISCUSSIONS

Candida albicans, a fungal pathogen causes various infections in man and represents a serious public health challenge with ever-increasing medical and economic importance owing to the high mortality rates and increased costs of treatment[11]. Emergence of antifungal drug resistance in candida albicansis a matter of great concern, because it is often resistant to many of the available antifungal drug like fluconazole, amphotericine-B, nad others azoles derivatives. So the need for new, low-cost, non-toxic the herbal compounds to treat infections caused by this

pathogen. Neem has documented antimicrobial properties, and can be effective against microbes like *Leishmania donovani*, against which it also has immunomodulatoryaction[12]. In our study it was shown for the first time as we know, that it has anti-candidal activity and can suppress growth and biofilm formation and act on cell wall of *Candida albicans*. The inhibitory compound was heat stable, since the extract was prepared by autoclaving. So it can be used safely and effectively in febrile states also. Toxicity also needs to be checked in different human cell lines in the form of further studies. All these are very important and interesting areas of further research.

CONCLUSION

Current study observe that neem leaf aqueous extract decreases the virulence factor of *candida albicans* strain, but to clearly establish the anti virulence activity of neem, further studies with large sample size is required.

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