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Inhibition of Growth, Biofilm and Lecithinase of *E. coli* by Extract of Neem Leaves (*Azadirachta indica*)

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A B S T R A C T

Escherichia coli is a Gram negative bacillus belonging to Enterobacteriaceae family, causing a plethora of human infections like Urinary tract infection (UTI), meningitis, diarrhoea and others. It is often resistant to multiple antibiotics. High cost of antibiotics, cost of prolonged hospital stay and refractoriness to drugs necessitates the invention of new, natural and low-cost compounds that can be effective against it. We here report the efficacy of extract of Neem leaves in inhibiting growth, bioflm formation and phospholipase (lecithinase), an important virulence determinant of *E. coli*. Furthermore, the extract was completely non-toxic to humnan RBCs and WBCs.

Introduction

Escherichia coli is a Gram negative bacillus causing infections in man like UTI and diarrhoea(1).It is frequently multi-durg resistant and about 40-60% of *E. coli* isolates can be positive for Extended spectrum beta lactamase, making treatment very difficult(2). Besides drug resistance, treatment of complicated infections due to specific types of E. coli, like Enterohemorrhagic *E. coli*, can be quite exorbitant and taxing for the poor patient(3). Phospholipse is a important virulence factor of *E. coli* and helps in cellular signalling(4). These problems necessitate the use of newer natural, low-cost compounds and herbal extracts to kill this pathogen.

The acetone and methanolic extracts of bark and leaves of Neem plant (*Azadirachta indica*) are very popular as traditional treatment in India , and have been found in studies to be cidal against microbes like *Pseudomonas aeruginosa*, *Staphylococus aureus* and *Candida albicans* (5). Keeping all these things in mind, our study was aimed at testing the efficacy of extract of Neem leaves against *Escherichia coli*, and to test its host cell toxicity.

Materials and Methods

This was a laboratory based observational study, carried out in the Department of

Microbiology in the institute as a institutional project, from January 2015 to November 2015.

Clearance of institute ethics commitee was sought and obtained prior to the study.

Preparation of Extract

Neem leaves were obtained from trees and plants in the hospital and residential complex. Tests were done with mature leaves as well as young leaves. In 2 sets of experiments, 4 gram and 8 grams of torn, smashed and dried neem leaves were added to 100 ml of Peptone water medium, and autoclaved at 121 deg. C at 15 lbs/in^2 pressure for 15 minutes. Ten (10) clinical isolates of *E. coli* were randomly selected for the study. 2 colonies of the bacterial isolates were suspended in : (a) Peptone water, and (b) Peptone water with neem extract at the concentration mentioned. The tubes were incubated at 37 deg. C overnight and then 1 loopful from each tube was subcultured on Egg yolk agar prepared in the lab (Nutrient agar 90 ml + egg yolk obtained by cleaning outer egg shell by alcohol, 10 ml). The plates were incubated at 37 deg. C overnight, Next day, colonies were observed for colony count reduction, and presence and alteration of phospholipase, protease and lipase activities.

Phospholipase (lecithinase) activity was defined as zone of opalescence (haziness) around colonies of egg yolk agar, while protease was denoted by zone of clearing around the colonies. Lipase activity was detected by appearance of pearly shine on surface of colonies on Egg yolk agar. Z TEST OF significance was done to test significant reduction in colony count(6).

For testing biofilm activity, test tube method was used, in which tubes containing *E. coli* in (a) Peptone water, and (b) Peptone water with neem, were taken and their liquid contents were disposed off. Tube were washed for 1 minute with sterile normal saline (0.9%), and 0.5% aqueous safranine was added to each tube, and kept for 1 minute. Then tubes were again washed thrice with normal saline, and placed in inverted position for visual observation of stained biofilms.

For testing toxicity of extract, 1 drop of extract was mixed with 1 drop each of (a) Normal saline, and (b) buffy coat of normal human blood (collected in lab for other tests, after tests were done). All test were done three times.

Results and Discussion

Neem extract, at 4 gram%, inhibited phospholipase and colony count (non-significant, $p>0.05$) of e. COLI on Egg yolk agar but not its biofilm. At 8 gram% concentration, however, there was marked reduction in colony count ($p <0.05$) and inhibition of its lecithinase activity. Protease was not shown by any *E. coli* isolates. Lipase of *E. coli* was not inhibited. Young neem leaves were found to be superior to mature leaves in antibacterial activity, in this regard. Biofilm of *E. coli* was also strongly inhibited at 8 gram% neem leaf concentration.

The extract was non-toxic as tested on human RBC and WBC.

Escherichia coli is a smart Gram negative pathogen causing various infections in man(1). Drug resistance in *E. coli* is a matter of great concern, because it is often resistant to many of the available antibiotics like fluoroquinolones and beta-lactams(7). So the need of the hour is the synthesis and availability of new, low-cost, non-toxic

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 immunomodulatory action(8). It also has marked acivity against *Enterococcus fecalis* and *S. aureus*(9). In our study it was shown that it has anti-*E.coli* activity and can suppress growth, lecithinase and biofulm formation by the bacterium. We also show for the first time that young neem leaves have superior effect on *E. coli* than mature ones. The effect due to the compounds was heat stable, since the extract was prepeared 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.

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