

Effect of *Azadirachta indica* extract on phagocytic activity against the common clinical pathogen

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ABSTRACT

Antimicrobial resistance exhibit by pathogenic microorganisms has encouraged more biocompatible and eco-friendly approaches to screening of herbs for their prospective antibiotic activity to manage and control infectious diseases. Herbal plants play a paramount role in the complementary medicines worldwide because of their many health benefits with no side effects. The objective of this study was to determine in vitro effect of *Azadirachta indica* (neem) on phagocytic activity against the common clinical pathogen *Staphylococcus aureus*. The phagocytic activity was determined by employing slide method. In this method, number of ingested cells was found to be 60 out of 100 after 1.5 hour incubation of blood sample with *Staphylococcus aureus* and *Azadirachta indica*. *Azadirachta indica* showed enhanced phagocytic activity as compared to control blood sample without the herb. This suggests that diet or drug supplemented with *Azadirachta indica* (Neem) is likely to augment the immune system and reduce the growth of pathogenic bacteria like *Staphylococcus aureus*.

Keywords

Phagocytic activity, *Azadirachta indica*,
Staphylococcus aureus.

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INTRODUCTION

Presently, worldwide there is an increase in diseases especially infectious diseases that require efficient body defense mechanism to control them. This can be achieved through the process of immunomodulation. Immunomodulation is basically the modification of immune response which decrease or increase the immune reaction to a desired level (1). Bricknell and Dalmo (2) defined the immunomodulant as “a naturally occurring compound that modulates the immune system by increasing the host’s resistance against diseases that in most circumstances are caused by pathogens”. A variety of allopathic drugs/medicines are used to modulate the immune system. However, often these are associated with antimicrobial resistance and undesirable drug reactions. Numerous infectious diseases have been

treated with herbal medication all over the history of mankind (3). These herbs are employed as immunostimulants to provide alternative prospective to conservative chemotherapy for a range of diseases particularly in relation to host defense mechanism (4). The majority of peoples in developing and under developing countries depend on herbal remedies to meet their health needs especially in those cases where synthetic medicines cannot provide relief from disease (5). In particular, infections caused by common clinical pathogen such as *Staphylococcus aureus* could be controlled through immunomodulatory herbal agents such as *Azadirachta indica* (neem). *Staphylococcus* infections ranges from mild to life threatening (6) and cause a variety of community and hospital acquired infections

including skin abscess (7), food poisoning, pneumonitis etc (8). *S. aureus* clones that resist methicillin (methicillin-resistant *S. aureus*, MRSA) disseminated and cause medicinal and public health problems worldwide. These strains are not only resistant to methicillin but also resistant to all other beta lactam drugs such as cephalosporins (9, 10). *S. aureus* is a classic opportunistic pathogen in this way because it takes benefit of breach skin or other entry sites to cause an infection.

Azadirachta indica is one of the most promising medicinal plants having a wide spectrum of biological activity, well known for its antimicrobial and insecticidal properties. *Azadirachta indica* is an ever green and fast growing tree belonging to family Meliaceae (11). All the parts of *A. indica* tree are frequently used in conventional household medication against a range of human diseases for centuries. Millions of people cleaned their teeth with neem twigs, skin disorders with neem leaf juice, neem tea as a tonic and placed neem leaves in their beds, cupboards, books etc to keep away troublesome bugs. Additionally, neem leaves have been accounted to possess immunomodulatory, enhanced phagocytic activity, anti-inflammatory, and antihepatotoxicity activity (12). The undertaken study, therefore, evaluated the effect of *Azadirachta indica* on phagocytic activity against the clinical pathogen.

MATERIALS AND METHODS

Test Microorganism: Human pathogenic bacteria *Staphylococcus aureus* were collected from the clinical laboratory in Karachi.

Preparation of Herb Extract: Extract was prepared by mixing 30 gram of *Azadirachta indica* herb in 100 ml sterile distilled water. The extract is then left for 24 hours. The herb solution is blended next day and filter through whatsmann no. 1 filter paper and store for further use.

Preparation of Bacterial Suspension: Human pathogenic bacteria *Staphylococcus aureus*, was cultured on nutrient agar media. These bacterial colonies were harvested from the agar plates, washed twice in

phosphate buffer saline and the concentration was standardized to 1×10^6 CFU/ml.

Test Blood: Blood samples were taken from the young individual. Blood was drawn from the arm and collected in tube. The tube is incubated with *Azadirachta indica* for the phagocytic activity.

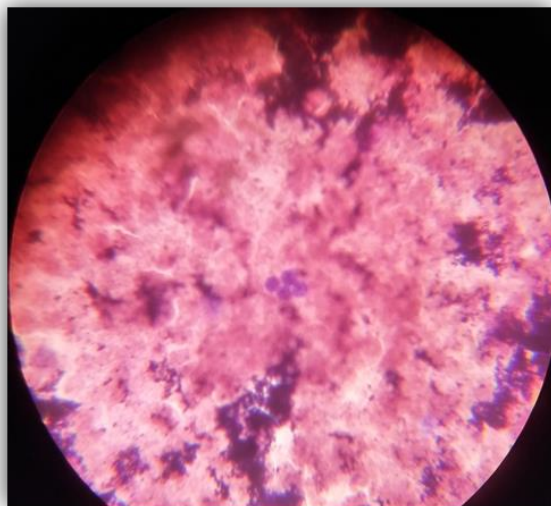
Phagocytic activity by slide method: 1ml fresh blood sample was placed in a tube with EDTA. 100ul of bacterial suspension and 100ul of herb extraction, both were mixed with the blood in the test tube. The mixture was incubated for 1 hour & 30 minutes in the incubator at 37°C then 1 to 3 drops of mixture were spread on the slide and stained by field staining method. Now to determine the phagocytosis of bacterial species, the number of ingested bacterial cells was counted per 100 of white blood cells (this include both phagocytic and non phagocytic cells).

RESULTS AND DISCUSSION

Continuously emerging infections of *Staphylococcus aureus* are problematic and increasing day by day due to extensive use of antimicrobial agents. These bacteria have acquired resistance against antibiotics like methicillin resistant *Staphylococcus aureus* (13). Emerging vancomycin intermediate *Staphylococcus aureus* strains also cause significance concern in the medical community (14). Remedial plants play a critical role in many countries for health needs. Plants and their products have been used safely for the treatment of medical problems. *Azadirachta indica* and neem leaves were used to demonstrate the immunomodulatory activity. Result obtained by the phagocytic activity of aqueous extract of *Azadirachta indica* against the clinical isolate (*Staphylococcus aureus*). The method employed was slide method (Figure 1). In this method by the use of neem aqueous extract, the ingested cells or non ingested cells were determined. The table below shows the result of slide method in which number of ingested cells were 60 and non ingested cells were 40. Neem extract inhibit the growth of organism and enhance the activity of immune system.

Table I: Phagocytic activity by slide method

Type of blood sample	No of ingested phagocytic cells	No of non-ingested phagocytic cells	Phagocytosis %
<i>Azadirachta indica</i> (Neem)	60	40	60%
Control	19	81	19%

**Figure 1:** Phagocytic cells with ingested bacteria observed in field staining.

Herbal based antibiotic compounds have massive therapeutic prospective as they can provide health benefit without any side effects that are frequently linked with synthetic medications. *Azadirachta indica* leaves possessed good antibacterial activity and is very useful in the health care (15). Leaf extracts of the neem has greatest activity and has the strongly inhibition activity (16). Plants are the significant source of potentially functional structures for the development of new chemotherapeutic agents. Enormous research has been done and many research reports are available on the antifungal, antibacterial and anti-inflammatory properties of plants (17). Nowadays scientists focus on the herbal medicines because it has minimal side effects as compared to antibiotic drugs and also gives promising results. Supplementation of particular immunomodulant such as neem leaf can be used in diet and can replace the antibiotic/antimicrobial agent to improve health and also reduce costs.

CONCLUSION

In the present era, herbal medicines are making a place in the pharmaceutical industry due to its higher antimicrobial activities against different pathogens and toxins. Scientists are focusing in different herbs as the research giving an evidence of their antipathogenic activity and reliability against different diseases. Herbal medicines usually have higher benefits and less side effects which making them more reliable among different synthetic medicines.

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