Bioenhancing Effect of Cow Urine Distillate and Pepper Extract on Antibacterial Activity of Azadirachta Indica Leaves

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ABSTRACT

Bioenhancers are drug facilitator which do not show the typical drug activity but in combination to enhance the activity of other molecule in several way including increase the bioavailability of drug across the membrane, potentiating the drug molecules by conformational interaction, acting as receptor for drug molecules and making target cell more receptive to drugs and promote and increase the bioactivity or bioavailability or the uptake of drugs in combination therapy. The objective of the present study was to evaluate the antibacterial and activity of combination in Azadirachta indica extract with cow urine distillate and pepper extract against common pathogenic bacteria, a causative agent of watery diarrhea. It has been found that Indian indigenous cow urine and its distillate also possess bioenhancing ability. Bioenhancing role of cow urine distillate (CUD) and pepper extract was investigated on antibacterial activity of ethanol extract of Azadirachta indica. Antibacterial activity of ethanol extract neem alone and in combination with CUD and pepper extract were determined the ATCC strains against Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and E-coli by cup plate diffusion method. Ethanol extract of neem has showed more effect on P. aeruginosa, E-coli than S. aureus and K. pneumonia with combination of CUD and pepper extract. CUD and pepper did not show any inhibition of test bacteria in low concentration. The antibacterial effect of combination of extract and CUD was higher than the inhibition caused by extract alone and is suggestive of the bioenhancing role of cow urine distillate and pepper. Moreover, inhibition of test bacteria was observed with less concentration of extract on combining with CUD.

KEYWORDS: Bioenhancer, cow urine distillate, pepper extract, Azadirachta indica, diffusion method.

Introduction

The knowledge on the use of bioenhancer is new to modern science but it was very familiar as yogvahi in Ayurveda. Yogvahi was used to enhance the bioavailability, tissue distribution, and efficacy of drugs, especially those with poor oral bioavailability and also decreasing the adverse effects (Randhawa G and Kullar SR, 2011). Bioenhancer is those substances which increase the efficacy of drug activity but may or may not show the any pharmacological effect. Such a bioenhancer have been earlier isolated from the plant source such as piperine, garlic, drumstick pods, licorice, turmeric, grape fruit, citrus fruits, black cumine (Singha et al., 2009).

Cow is equated to mother in the Indian tradition and her urine panacea of all diseases. Cow urine is a divine medicine and is used for treatment of diabetes, blood pressure, asthma, psoriasis, eczema, heart attack, blockage in arteries, fits, cancer, AIDS, piles, prostrate, arthritis, migraine, thyroid, ulcer, acidity, constipation, gynecological problems (Mohanty et al., 2014). In India, drinking of cow urine has been practiced for thousands of years. One of the forms of cow urine with popular use nowadays is cow urine distillate, a transparent liquid obtained when cow urine is subjected to distillation. The distillate is having more acceptability than crude cow urine and shows activities of crude cow Urine (Rakshitha et al., 2010; Surabhi K et al., 2011). Cow urine distillate is more effective as a bioenhancer than cow urine, and increases the effectiveness of antimicrobial, antifungal and anticancer drugs. It also increases the activity of gonadotroin releasing hormone conjugate with bovine serum albumin (GnRH–BSA) and zinc (Randhawa GK., 2010). Cow urine/gomutra has been elaborately explained in Ayurveda as effective medicinal substance with innumerable therapeutic properties. Cow urine has bioenhancing activity for rifampicin, and also increases action against E. coli (5-7 fold) and gram positive bacteria (3-11 fold). Cow urine distillate is more effective as a bioenhancer than cow urine. It enhances the transport of antibiotics like rifampicin, tetracycline and ampicillin across the gut wall by 2-7 folds (Mekala et al., 2012). It also enhances the potency of taxol against MCF-7 cell lines (US Patent No. 6,410,059).

Few studied reported Pepper extract contain piperine act as a bioenhancer to improve the bioavailability of...
antibiotic, nutritive substance. Piperine act by suppressing P-gp and cytochrome P450 enzyme, which counteract the metabolism of antibiotic via these proteins. It also decreases the intestinal production of glucuronic acid, thus allowing more substances to enter the body in active form (Randhawa GK et al., 2011).

Nature has provided a complete store house of remedies to cure all ailments of mankind. The natural or herbal remedies are still the backbone of medicines. Phytotherapy is a medicinal practice based on the use of herbal plants and their extracts. These herbs or plants and their active ingredients are used in traditional herbal remedies. The easy availability, low cost and negligible side effects, natural products are popular in the nowadays in the world (Asif M., 2012). Medicinal plants have been extensively studied as an alternative treatment for disease in order to overcome the problem of antibiotic resistance by pathogenic organisms. Number of plant have been proven by rigorous science or approved by regulatory agencies such as the United State Food and Drug administration, European Food Safety Authority to have medicinal value of Azadirachta indica is inclusive (Timothy et al., 2011). Azadirachta indica (neem) is used in traditional medicine as a source of many therapeutical agents in the Indian culture and grows well in the tropical countries. Its twigs provide a chewing stick and are widely used in the Indian sub-continent earlier studies on neem have showed that it contains active substances with multiple medicinal properties (Maragathavalli et al., 2012).

The purpose of the present study was to investigate the antimicrobial activity of neem leaves against human pathogenic E-coli, K. pneumoniae, Staphylococcus aureus and P. aureoginosa with combination of selective recommended natural bioenhancer.

Materials and Methods

Collection and identification of plant: The plant material was collected during June 2014 from B.G Nagara, Nagamangala taluk of Mandya district, Karnataka, India. The plant sample was identified by Dr. Anney Gowda H, Head of department, Dept. of Pharmacognosy, SAC College of pharmacy, Mandya, Karnataka.

Method of extraction: Dried seeds of Piper nigrum were purchased from the local market of Bellur and dried at 40 °C for 4 hours in hot air oven, finely powdered and packed in self-sealing plastic cover until further use. Fresh leaves of Azadirachta indica were collected from BG Nagara in Mandya district. Leaves were separated, dried in cool place of laboratory for 5 days, finely powdered and packed in self-sealing plastic cover until further use. Dried powders were extracted with 99% ethanol using a Soxhlet extractor apparatus. The 100 g power was put in Soxhlet thimble and put into a Soxhlet thimble tube. 1000 mL of ethanol was added to a Soxhlet flask, and then extracted at 60 °C until the extract was not clear or about 8 hour. The ethanol was removed under pressure using a rotator evaporator. Then dried residue crude extract were stored in dark bottle a 4 °C (Ekaidem et al., 2010).

Preparation of media plate: Suspend 38g Muller Hinton agar (Hi-media) powder in one liter of distilled or deionized water in a conical flask and sterilize at 121 °C (15 lbs. of pressure) for 15 minutes. Cool to 40-45 °C and pour the molten agar to a depth of ¾ of the sterilized plate in a laminar flow. Keep the Petri dishes horizontally until the medium completely solidifies. Turn dishes upside down and stack them up for storage (Vinoth et al., 2012).

Source of microorganisms: Strain of E-coli, K. pneumoniae, Staphylococcus aureus and P. aureoginosa were used as tested organisms in all antibacterial assays. These ATCC grade organism stains were taken from Department of Microbiology, Sri Adichunchanagiri Institute of Medical Sciences. These organisms were selected because they are among many pathogens often implicated in food borne outbreak in the world. Different strains of bacteria were streaked onto Tryptone Soy agar (TSA) to obtain pure isolated colonies, following a standard aseptic technique and the four-way streak plate inoculation. Once the isolated colonies were obtained, the bacterial strains were enumerated with Mueller Hinton Broth (MHB) for the next step of the experiment, the antimicrobial assay (Dharma, 2005).

Cow urine distillate: CUD was purchased from the Maa Gou Product Pvt. Ltd., Bengaluru road, yeshvathapura, Karnataka.

Antimicrobial screening

Cup plate diffusion method: The antibacterial activity of the extract was determined by using the agar well diffusion technique. Well of 9 mm were bored in sterile Mueller Hinton agar plates (Himedia, Mumbai) and inoculated with 24 hours old broth cultures of test bacteria using sterile cotton swab. The neem extract (50 mg/mL of 10% DMSO), CUD, pepper extract (50 mg/mL of 10% DMSO) Control (10% DMSO) and neem extract- CUD and neem extract- pepper extract combination (1ml extract (50 mg/mL) and 1 mL CUD mixed; extract concentration in combination- 25 mg/mL) were added into the respectively labeled wells. The plates were incubated at 37 °C for 24 hours in upright position and mean value of zone of inhibition was recorded. Measured inhibition zone were recorded as mean diameter in cm. The experiment was carried three times to get concordant result (Tambekar and Kerhalkar, 2006).

Results and Discussion

The result of this study showed that the ethanolic leaf extract of Azadirachta indica contains saponins, tannins, glycosides, alkaloids, terpenes, flavonoids and reducing sugar these secondary metabolites could be responsible for its activities against Staphylococcus aureus, Escherichia coli, Klebsilla pneumoniae and Pseudomonas aregunisa. The wide use of Neem plant is attributable to the presence of these bioactive compounds which may explain its diverse traditional usage against various ailments. Where aqueous extract of Azadirachta indica leaf contains penosis and carbohydrate in addition...
to those chemical constituents detected. Anthraquinones was not detected in both solvents (Vinoth et al., 2012).

In this study, bioenhancing effect of CUD and pepper on antibacterial activity of ethanolic extract was tested. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative. Ethanolic extract (50mg/ml) has shown more effect on E. coli followed by P. aeruginosa, K. pneumoniae and S. aureus. CUD caused no inhibition of test bacteria. No inhibition of test bacteria was observed in case of control (10% DMSO). The antibacterial effect of combination of extract and CUD was higher than the inhibition caused by extract alone. In combination trial also, E. coli was more inhibited followed by P. aeruginosa and S. aureus. In combination with CUD, extract at concentration 25 mg/mL has shown more inhibition of test bacteria as compared to inhibition produced by ethanolic extract at 50 mg/mL concentration.

Antibacterial activities of neem extract observed alone and combination with 500 mg/mL extract in 10% DMSO solution. 10% DMSO solution act as negative control. Test samples in all concentrations and negative control showed no activity in all four organisms. This results clearly indicated the bioenhancing activity properties was in the Pepper> CUD. Zone of inhibition in P. aeruginosa was increase more than 30% and similarly zone of inhibition of E. coli was more than 25%.

TABLE 1

Antibacterial activity of extracts, and CUD.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Zone of inhibition in cm</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>10% DMSO</td>
<td>0.0</td>
</tr>
<tr>
<td>CUD</td>
<td>0.0</td>
</tr>
<tr>
<td>Neem extract</td>
<td>1.8 ± 0.096</td>
</tr>
<tr>
<td>Neem extract + CUD</td>
<td>2.3 ± 0.092</td>
</tr>
<tr>
<td>Neem extract + Pepper extract</td>
<td>2.4 ± 0.089</td>
</tr>
</tbody>
</table>

Fig. 1. Antibacterial activity of extract and CUD.

Conclusions

From this work, result marked inhibition of test bacteria in case of combination trials than single treatments thus suggesting the bioenhancing role of pepper and cow urine distillate. Thus, formulations containing CUD could be useful in reducing the dosage of drugs and to treat the infections.

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References


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