International Multidisciplinary Research Journal 2011, 1(9): 12-15
ISSN: 2231-6302
Available Online: http://irjs.info/

INTERNATIONAL MULTIDISCIPLINARY RESEARCH JOURNAL

Synergistic interaction between chemotherapeutic analogues and chloroform fruit extract of *Acacia arabica* (L.) willd var. indica against enterotoxigenic *Escherichia coli*

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Abstract

Diarrheal infections caused by MDR *E. coli* have been recognized as one of the most serious health problems. Bioactive principles present in plant extracts has ability to sensitize chemotherapeutic analogues which gets completely ineffective against pathogen. Synergistic interaction between chloroform fruit extract of *Acacia arabica* Lam. Willd var i*ndica* and five different chemotherapeutic analogues (Tetracycline, Erythromycin, Chloramphenicol, Ampicillin and Penicillin G) provides a promising approach for treatment of diarrheal infection associated with enterotoxigenic *E. coli. In vitro* synergistic efficacy between plant extracts and antibiotics were performed by well-diffusion method. Results showed that interaction between chloroform fruit extract/tetracycline, chloroform fruit extract/erythromycin and chloroform fruit extract/chloramphenicol were synergistic and additive however combinations between plant extracts and ampicillin showed no synergistic effect against ETEC (Standard) strain. Synergism was also not observed on concurrent administration between extract and penicillin G against ETEC (Environmental isolate). Despite of two negative results clear synergistic/additive interaction was verified in all combinations used against tested organism.

Keywords: MDR *E.coli*, Synergistic interaction, *Acacia arabica* Lam. Willd, chemotherapeutic analogues

INTRODUCTION

Diarrhea associated with various multi -drug resistant serotypes of ETEC Enterotoxigenic *E. coli* is one of the major problem faced by both developed and developing countries and is one of the major cause of infant morbidity since the bacterial infection causes sever dehydration after colonization in intestine. Adverse conditions frequently occur due to malnutrition among infants in underdeveloped countries. Poor sanitary conditions, lack of hygiene as well as lack of proper control strategies and absence of drugs to control infections are also major factors responsible for diarrheal infections among young children. Execution of disease control strategies plays a very important role to control such type of endemic diseases caused by *E. coli* (1). ETEC contain two major enterotoxins Heat stable (ST) and Heat Labile (LT) and horizontal transfer of such plasmid mediated enterotoxins were reported (2),(3). Due to indiscriminate use of antibiotics emergence of drug resistant strains became a major problem world wide and makes treatment complicated and most old and cheap antibiotics such as penicillin, Tetracycline and Erythromycin gets worthless (4).

India is always having a wide range of medicinal plants that are principally used to cure treatment of most of the bacterial and non bacterial ailments for long history. There are more than 7000 species of medicinal plants reported through out the world but only 1500 of plants are used in Ayurveda, Siddha and Unani medicines (5). Due to contemporary problem of multi drug resistance it is necessary to investigate an effective bio herbal formulation in order to treat diarrheal infections associated with Enterotoxigenic *E.coli.* In rational drug therapy combined administration of two or more drug to cure ailment is considered to be obligatory (6). Concurrent administration of herbal extracts and chemotherapeutic analogue is a novel concept and could be beneficial (synergistic or additive) or deleterious (antagonistic or toxic outcome) (7). It is necessary to understand the mechanism of synergism for development of a new pharmacological agent to treat diseases (8), (6). Phytochemicals in combination with antibiotics are capable to enhance total biological activity of ineffective antibiotics to control targeted disease causing organism (9). Synergistic antimicrobial potency between tea and antibiotics against enteropathogenic *E.coli* (EPEC P2, 1265) was reported (10).

Acacia arabica (L.). willd sub sp. *indica* or *Acacia nilotica* (L.) willd sub sp. *Indica* is a wild, moderate size leguminous plant belonging to family Fabaceae and its leaves, bark, gums and fruits are used for various medical purposes including gonorrhea, dropsy, soar throat, eye infections as well as for treatment of intestinal disorders (11-13).

The main aim of the study is to investigate *in vitro* synergistic interaction between crude chloroform fruit extract of *Acacia arabica* Lam. Willd var i*ndica* with five different chemotherapeutic drugs against two different enterotoxigenic strain of *E. coli.*

METHODOLOGY

Collection of plant material and preparation of extract

Fresh immature pods of *Acacia arabica* was collected during February – March month from road side of Bhilai-Durg region. Fruits/ pods were thoroughly washed under tap water, cut into small pieces and shade dried for 2-3 weeks. Dried plant material was finely

Received: June 17, 2011; Revised September 15, 2011; Accepted September 15, 2011.

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pulverized using domestic mixer for extract preparation. Chloroform fruit extract was prepared by soaking 7.5 g of finely pulverized fruit in exactly 100 ml of Chloroform and shaken for 24 hrs in rotary shaker at 120 r.p.m. and concentrated to dryness at room temperature to obtain crude extract (14).

Collection of Bacterial Strains and preparation of inoculums

Enterotoxigenic *E. coli* strain *E. coli* O78:K80:H11 was obtained from IMTECH, Chandigarh carry ST and LT enterotoxins. In addition, environmental enterotoxigenic *E. coli* carrying LT enterotoxin was also obtained from St.Thomas College and various biochemical tests were performed to detect its authenticity. The media used for the drug sensitivity purpose were Muller- Hinton's agar (Hi-media) and Muller- Hinton's broth (Hi-media). Cultures were adjusted according to Mc Farland turbidity 0.5 standards in MH- Broth.

Antibacterial Drugs used

Five different drugs were used to investigate synergistic interaction with chloroform fruit extract which includes three protein synthesis inhibitor Tetracycline, Erythomycin, Chloramphenicol and two cell wall synthesis inhibitors Ampicillin and Penicillin G.

Antibacterial Susceptibility Test

Antibacterial activity of extract, antibiotics and combination of antibiotics +extract was determined by well-diffusion method (15). About 100 µl of Muller-Hinton's broth containing test organisms were aseptically spread over Muller-Hinton's agar plate and spread uniformly with colon swab and allowed to dry for half an hour. With a sterile well cutter of 6 mm diameter wells were cut in all petriplates. About 100 µl of five different antibiotics of 0.2 mg/ml were added to each well individually as well as in combination with plant extracts to estimate additive activity of combination to inhibit test organism. Estimation of synergistic / antagonistic activity was performed by measuring zone diameter of plant extract, antibiotics and combination of both (extract +antibiotics) on next day after incubation under standard condition for 24 hrs at 37^0C. All experiments were performed in triplicates and average of each observation was recorded.

RESULTS AND DISCUSSION

The results of crude chloroform fruit extract of *Acacia arabica* (L.) Willd on tested organism was shown in Table: 1. Results obtained from the extracts showed effective growth limiting response against ETEC on the basis of inhibition zone diameter. Fruit extract of *Acacia arabica* was reported to be rich in tannin, flavonoids and cardiac glycosides which are mainly associated with antibacterial activity (16).

Table: 1. Determination of Inhibition Zone Diameter of Chloroform Extract (Inhibition Zone Diameter in mm)

	500 mg/ml	250 mg/ml	125 mg/ml	62.25 mg/ml	31.32 mg/ml
ETEC (Environmental Isolate)	10 ±0	8±0	4±0	0±0	0±0
ETEC (Standard)	8.6±0.6	7.3±0.6	6±0	0±0	0±0

*ETEC= Enterotoxigenic *E. coli*

The data mentioned in Table: 2 represent that ETEC (Environmental Isolate) showed drug resistance to all five antibiotics whereas ETEC (Standard) strain showed sensitivity to tetracycline and chloramphenicol and resistant against erythromycin, ampicillin and penicillin G.

Table: 2 Drug Resistance / Sensitivity pattern of ETEC Strains

	Tetracycline	Erythromycine	Chloramphenicol	Ampicillin	Penicillin G
ETEC (Environmental Isolate)	R	R	R	R	R
ETEC (Standard)	S	R	S	R	R

*ETEC= Enterotoxigenic *E. coli*, R- Resistant, S- Sensitive

On combined administration of chloroform fruit extract and tetracycline as represents in Table: 3 showed strongest synergistic and additive antibacterial activities was recorded with enlargement of zone of inhibition against both ETEC (Environmental isolate) and ETEC (Standard) strains respectively.

Table: 3 Determination of Synergism between Chloroform Fruit Extract and Tetracycline

	Concentration of fruit extract (in mg/ml)	Concentration of tetracycline (in mg/ml)	Inhibition zone diameter of Fruit extract (in mm)	Inhibition zone diameter of Tetracycline(in mm)	Inhibition zone diameter of fruit extract +Tetracycline (in mm)
ETEC (Environmental Isolate	500 mg/ml	0.2 mg/ml	10 ±0	0±0	20±0
ETEC (Standard)	250 mg/ml	0.2 mg/ml	7.3±0.6	21.3±0.6	23.3±1.73

*ETEC= Enterotoxigenic *E. coli*

Synergistic activity was reported between methanolic flower extract of *T. populnea* and oxytetracycline against *E. coli* ATCC 11775 (17). Combined antibacterial efficacy between chloroform fruit extract and erythromycin in Table: 4 & with chloroform fruit extract with chloramphenicol in Table: 5 also indicate strongest synergistic interaction to inhibit test organism therefore suggests that concurrent administrative approach of two drugs at same time can effectively alter microbial infections in much better way in comparison to administration of a single drug.

Table: 4 Determination of Synergism between Chloroform Fruit Extract and Erythromycine

	Concentration of fruit extract (in mg/ml)	Concentration of Erythromycin (in mg/ml)	Inhibition zone diameter of Fruit extract (in mm)	Inhibition zone diameter of Erythromycin (in mm)	Inhibition zone diameter of fruit extract +Erththromycin (in mm)
ETEC (Environmental Isolate	500 mg/ml	0.2 mg/ml	10 ±0	0±0	16.6±0.6
ETEC (Standard)	250 mg/ml	0.2 mg/ml	7.3±0.6	9.3±0.6	21.3±0.6

*ETEC= Enterotoxigenic *E. coli*

Table: 5 Determination of Synergism between Chloroform Fruit Extract and Chloramphenicol

	Concentration of fruit extract (in mg/ml)	Concentration of Chloramphenicol (in mg/ml)	Inhibition zone diameter of Fruit extract (in mm)	Inhibition zone diameter of Chloramphenicol (in mm)	Inhibition zone diameter of fruit extract +Chloramphenicol (in mm)
ETEC (Environmental Isolate	500 mg/ml	0.2 mg/ml	10 ±0	10.6±0.6	16±0
ETEC (Standard)	250 mg/ml	0.2 mg/ml	7.3±0.6	17.3±2.4	21.3±0.6

*ETEC= Enterotoxigenic *E. coli*

On concomitant administration of extract and ampicillin In Table: 6 synergistic inhibitory effect was observed against ETEC (Environmental isolate) but positive interaction was not experienced in ETEC (Standard) The effect of fruit extract on ETEC (Standard) was completely masked by the effect of ampicillin. Similar ineffective response was observed in ETEC (Environmental isolate) on parallel administration of extract and penicillin G in Table: 7 in which activity of extract was completely suppressed by activity of penicillin G. However, strongest synergism was recorded on joint administration of extract and penicillin G against ETEC (Standard).

Table: 6 Determination of Synergism between Chloroform Fruit Extract and Ampicillin

	Concentration of fruit extract (in mg/ml)	Concentration of Ampicillin (in mg/ml)	Inhibition zone diameter of Fruit extract (in mm)	Inhibition zone diameter of Ampicillin (in mm)	Inhibition zone diameter of fruit extract + Ampicillin (in mm)
ETEC (Environmental Isolate	500 mg/ml	0.2 mg/ml	10 ±0	0±0	16±0
ETEC (Standard)	250 mg/ml	0.2 mg/ml	7.3±0.6	0±0	0±0

*ETEC= Enterotoxigenic *E.coli*

Table : 7 Determination of Synergism between Chloroform Fruit Extract and Penicillin G

	Concentration of fruit extract (in mg/ml)	Concentration of Penicillin G (in mg/ml)	Inhibition zone diameter of Fruit extract (in mm)	Inhibition zone diameter of Penicillin G (in mm)	Inhibition zone diameter of fruit extract + Penicillin G (in mm)
ETEC (Environmental Isolate	500 mg/ml	0.2 mg/ml	10 ±0	0±0	10 ±0
ETEC (Standard)	250 mg/ml	0.2 mg/ml	7.3±0.6	0±0	18±0

*ETEC= Enterotoxigenic *E.coli*

The mode of action of combined administration of herbal extracts and antibiotics differs significantly than that same drug acting individually thereby overcomes MDR microorganisms which is helpful to isolate a single compound which has lost its importance due to drug resistant strains (18). *In vitro* synergism between plant extracts and drugs were reported to have inhibitory effect against multidrug resistant *E.coli* (19). Understanding the mechanism of synergism is mandatory for development of an effective chemotherapeutic agent to cure diarrheal infections associated with *E. coli.* The synergetic effect on combined administration between plant extracts and antibiotics against MDR bacteria leads to new way for treatment of infectious diseases (20), (21).

In conclusion, our results revealed in *vitro* synergistic interaction between five different drugs and chloroform extract of *Acacia arabica* (L.) Willd against enterotoxigenic *E. coli* which may be helpful to cure diarrheal infection.

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