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EFFECT OF ACTINOMYCETE EXTRACT ON EXPERIMENTAL MODEL OF ACUTE SYSTEMIC BACTERAEMIA OF VANCOMYCIN RESISTANT *ENTEROCOCCUS* IN SWISS ALBINO RATS

M.KALPANA DEVI[1] AND R.USHA*[1]

[1]Department of Microbiology, Karpagam University, Karpagam Academy of Higher Education, Coimbatore- 641 021, Tamil Nadu, India.

ABSTRACT

Actinomycetes provide important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances. The best actinomycetes isolate named as ACT 24 extract have antibacterial effect on the *Staphylococcus aureus* and *E.coli* with varying degrees in the range of 14-36mm. The isolate also shows good activity against tested organism *Vancomycin Resistant Enterococcus* (VRE) in the range of 26mm. This study is about the effect of ACT 24 against problematic organisms into the blood stream in Swiss albino rats. The present study was carried out in adult healthy Swiss albino rats. The control group shows no growth on enterococcal agar. In VRE induced group, the blood samples show more number of colonies on Enterococcal agar VRE were detected in almost all the organs of rat. ACT 24 treated rat organs were homogenised and plated on enterococcal agar, one or two colonies were observed and absence of apparent pathological changes. This study concludes with that ACT 24 extracts show good activity against VRE in rat model.

KEY WORDS: *VRE, Bacteraemia , Nosocomial Infection and Solvent Extract.*

R.USHA*

Department of Microbiology, Karpagam University, Karpagam Academy of Higher Education, Coimbatore - 641 021, Tamil Nadu, India.

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INTRODUCTION

The emergence of multidrug resistant during the past decade converted some enterococci from being difficult to treat and to being impossible to treat[1]. Since vancomycin became the agent of last resort for the treatment of infections caused by multidrug resistant *Enterococcus faecium*, it was under great concern that the first reports of *Vancomycin resistant enterococcus* in Europe began to appear in the mid 1980s[2]. Treatment of serious VRE infections is limited by the paucity of effective antibiotics[3]. VRE colonizes the gastrointestinal tract and it is likely that systemic bloodstream infections are the result of dissemination from the intestine[4]. It is widely assumed that antibiotic treatment, by eliminating commensal flora, opens intestinal niches and provides increased access to nutrients, thereby enhancing VRE survival and proliferation. Microbial extracts are widely used in human medicine, both prophylactically and therapeutically, for controlling diseases. Microbial natural products are the origin of most of the antibiotics on the market today. There is an alarming scarcity of new antibiotics currently under the development in pharmaceutical industry[1]. Actinomycetes provide important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances .In order to develop and establish the safety and efficacy level of a new drug, toxicity studies are very essential and no drug is used clinically without its clinical trial as well as toxicity studies [5] .Depending on the duration of exposure of the animals to the drug, toxicological studies may be classified as acute, subacute or chronic[6,7]. In acute toxicity studies, the animal is given a single dose of drug to determine the immediate toxic effect. Acute toxicity studies are commonly used to determine lethal dose (LD50) of a drug. Subacute toxicity studies are used to determine the effect of drug on the biochemical and haematological parameters of blood and any histopathological changes. Here, toxicological data helps to make decision whether a new drug should be adopted for clinical use or not[8]. Therefore, in connection of this objective, the present work was conducted to report whether the extract of actinomycetes have greater activity without any adverse effect systematically in the blood circulation.

MATERIALS AND METHODS

The present study was carried out with adult healthy Swiss albino rats (500g) with the approval of CPCSEA (KU/IAEC/Ph.D/140) in the Animal House, Karpagam University (Coimbatore), Tamil Nadu, India. The rats were examined to rule out the presence of any disease. The animals were housed in separate cages, supplied with food and water, and allowed to live in optimal conditions according to the hospital housing protocol of the Laboratory Animal Unit, Karpagam University.

Preparation of the solvent ACT 24 extract

ACT 24 isolates were isolated from mangrove soil which was inoculated into 1000ml of Starch casein broth and incubated at 30˚C for 10 days in shaking incubator. After incubation, the fermented broth were filtered and centrifuged at 14,000 rpm for 10 min. Filtrates were extracted with suitable solvent ethyl acetate and purified using column chromatography, ethyl acetate and methanol 95:5(v/v) mixture was used as an eluting solvent. Active fractions were preserved in a refrigerator until use[9].

In vitro study

VRE strain was isolated from clinical sample used in the study. A strain was freshly prepared before working by overnight incubation in VRE broth. Antimicrobial activity of ACT 24 extracts was measured against test pathogens namely *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and VRE by using the agar well diffusion method according to the procedure of La Fuente[10] with modification. The Minimum Inhibitory Concentrations (MICs) of extract (ACT 24) were determined by microdilution techniques in Muller Hinton broth. Chloramphenicol was used as reference antibiotic[11].

In vivo study

Acute toxicity studies for the determination of median lethal dose (LD50) according to OECD guidelines, 12 animals were used for each test. The animals were kept fasting for overnight but providing only water, after which the extracts were administrated orally at the dose of 5,10,15 mg/kg through oral gavage to three groups and observed for physical signs of toxicity for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administrated was assigned as toxic dose. If the mortality was observed only in 1 out of 3 animals, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose i.e. 1000 µg/kg. The LD50 was calculated by the method of Miller and Tainter[12].

Effect of solvent extract on translocation of VRE into different organs of rat

In vivo study was carried out on 12 adult healthy Swiss albino rats were divided into three groups of 4 rats each: Group 1: negative control group (received intragastric saline solution [5000µl/kg] daily); Group 2: positive control group (injected with VRE bacterial suspension containing 1.5 x 10^6 colony-forming units (CFU/mL) of intraperitonial injection at a dose of 5000µl /kg and then received intragastric saline solution daily; Group 3: injected with bacterial suspension containing 1.5 x 10^6 CFU/mL of VRE then injected with ACT 24 extract 5000µl/kg in oral[13].

Enumeration of viable bacteria in blood, liver, spleen, kidney and heart

Blood samples were obtained by eye puncture with a disposable heparinised syringe under ketamine – xylazine anaesthesia. After blood collection the animal were sacrificied and the spleen, liver, heart and kidney were dissected out. Each organ was homogenised and diluted in prereduced Hank's balanced salt solution supplemented with cysteine HCl 0.005% (HBSSs). The colon was aseptically dissected out, immediately transferred to an anaerobic chamber and its content were weighed before suspended in to HBSSs. The homogenate was serially diluted with same solution and counted by Haemocytometric method then plated on Enterococcosal agar with Vancomycin[1].

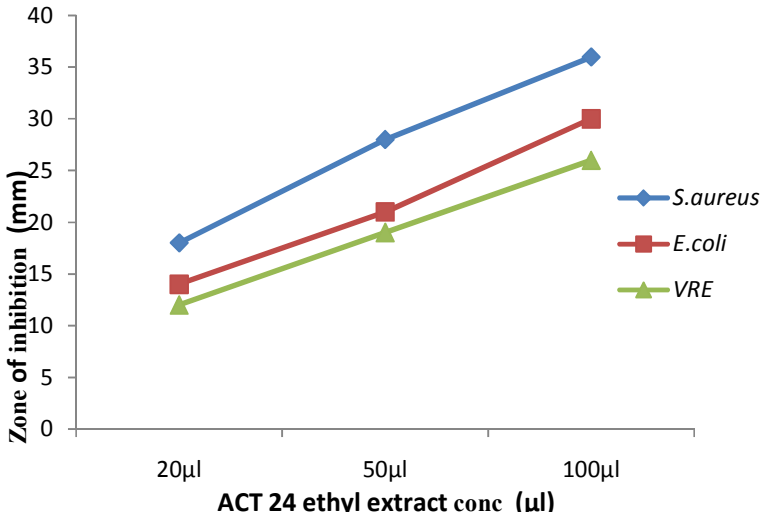
RESULTS

In vitro study

Sensitivity test

The obtained results include testing of whole ethyl acetate extract of ACT 24 against VRE. The results reveal that ACT 24 extract shows the activity with varying degrees in the range of 12-26mm (Graph-1).

Antibacterial activity of isolate against pathogens



Graph-1

Antibacterial activity of isolate ACT24 ethyl acetate extract shows good activity against Staphylococcus aureus (18-36mm), Escherichia coli (14-27mm) and VRE (11-24mm).

Staphylococcus aureus (ATCC 25923) and *Escherichia coli* (25922) show good activity with the zone of inhibition ranges 36mm and 30mm respectively. Well diffusion method is more sensitive than disc diffusion method[14]. The strain SRB25 was more similar to *Streptomyces* with antimicrobial property against multidrug.The active strain SRB25 shows almost the achieved results against panel of pathogens.MBT1 and MBT2 inhibited growth of E.coli ET8 most strongly when grown on routine MMagar[15].

Minimal inhibitory concentration of solvent extract

The Minimum Inhibitory Concentration (MIC) of extract was presented in Table 1. The MIC of the extract ACT 24 ranged from 2.25 to 5 mg/ml. MIC of the reference antibiotic chloramphenical shows 4.0mg/ml against VRE.

Table-1

Minimal inhibitory concentration of solvent extract of actinomycetes

Test organisms	MIC of ACT 24 (mg/ml)	MIC of Chloramphenicol
VRE	4.25mg/ml	4.0mg/ml
Staphylococcus aureus	2.5mg/ml	2.5mg/ml
Escherichia coli	3.25mg/ml	2.5mg/ml

VRE – Vancomycin Resistant Enterococcus

Table shows the minimal inhibitory concentration of solvent extraction of actinomycetes against Staphylococcus aureus (2.5mg/ml), Escherichia coli(3.25mg/ml) and VRE(4.0mg/ml)

The antibacterial activity produced by the extract was statistically significant (*P* <0.05 vs. the control). *Staphylococcus aureus* and *Escherichia coli* growth were minimized at 2.25 and 3.5mg/ml respectively. ACT 24 shows good activity against VRE at 4.25 mg/ml. The growth of the microorganisms is indicated by turbidity in the wells[16]. Hannah *et al.*[17] (2008) reported that crude extract was tested against *Staphylococcus aureus*, *Candida albicans*, *Bacillus cereus*, and *E.coli*. Notably, the observed MIC value for the solvent extract was higher than that of chloramphenicol.

In vivo study

Acute toxicity study

Infections caused by the genus *Enterococcus* (most notably *E. faecalis*, which accounts for 80% of all infections) include urinary tract infections, bacteremia, intra-abdominal infections, and endocarditis. Solvent extract was tested for the toxicity against swiss albino rat. No toxicity symptom was observed with the selected dosage level (Table 2).

Table-2
Shows toxicology study of different doses in swiss albino rat

Groups	Dose ml/kg	Mortality	Toxic symptoms
		Death/Treatment (NOA)	
Group I	Saline 10ml/kg	0/3	Nil
Group II	5mg/kg	0/3	Nil
GroupIII	10mg/kg	0/3	Nil
Group IV	15mg/kg	0/3	Nil

NOA : Number of animals, P <0.05 compared with control.

Table-2 Group-I-IV not shown any toxic symptoms and mortality up to 15mg/kg

At the dose level of 15mg/kg of ACT 24 treatment, the animals were showed no adverse effect of diarrhea, haematuria, twisting, colonic convulsion. The animals received dose upto 15 mg/kg orally was not found to cause any mortality and non-significant changes which were observed in wellness parameters used for evaluation of toxicity (Table 2). However, the low doses did not produce any pronounced effects. Thus, at normal therapeutic doses, crude extract is considered to as safe for long-term treatment[13].

Effect of solvent extract on translocation of VRE into different organs of rat

Control group
Saline injected rat Group 1 rat showed no growth on enterococcal agar.

VRE inoculated rat
Group 2 rat shows high mortality after 2 weeks. The morbitied rat was showed abcess in post thigh region, pyelonephritis and cloudy swelling of liver were seen. The blood samples show more number of colonies on Enterococcal agar (table-3). The inoculated VRE were detected in almost all the organs of rat. Similarly, reported that inoculated VRE was detected in all blood samples obtained from dead and dying rat.

Table-3
Translocation of intestinal bacteria into the blood, liver, spleen, kidney and heart of VRE inoculated rat.

Rat no	Number of organism (cfu/tissue sample)				
	Blood	Liver	Spleen	Kidney	Heart
1	3	3	2	67	45
2	63	10	3	65	67
3	88	12	15	54	78
4	32	7	17	65	34

P <0.01 compared with test extract; CFU: Colony forming Unit.

Table-3 more number of organisms were shows in blood of VRE inoculated rat: Liver and spleen shows one or two organisms

The bacterial counts were observed in blood and followed by liver, spleen, heart and kidney. However more number of organisms were detected in kidney and heart.VRE translocated and established the infection to all the mentioned above organs without solvent extract treatment. Statistically it is not significant (*P* <0.01) when compared with test.

ACT 24 orally treated rat
Group 3 all the four animals spleen, liver, kidney and heart were dissected out, each organ homogenised and plated on enterococcal agar. Below six colonies only observed and the absence of apparent pathological changes (Table-4). No difference was detected after 2 weeks.

Table-4
Translocation of intestinal bacteria into the blood, liver, spleen, kidney and heart of antimicrobial compound treated rat.

Rat no	No of organism (cfu/tissue sample) in rat organ				
	Blood	Liver	Spleen	Kidney	Heart
1	2	NG	NG	4	4
2	4	NG	2	2	5
3	5	NG	1	3	2
4	NG	1	NG	NG	1

NG: no growth, P <0.05 compared with control.

Table-4 Antibacterial treated group shows less than five organisms in blood and other organs.

The antibacterial activity produced by ethyl acetate extract was statistically significant
(*P* <0.05) when compared with control. Growth inhibition level is significantly different as positive control. The result of the *in vivo* evaluation of the ACT 24 extract on infected rat showed the extract was active at 5mg/kg by showing less number of organisms in the different organs (Table 4).

DISCUSSION

Bacterial diseases are responsible for heavy mortality in human. Several aquaculture industries use the chemotherapy for the treatment of various microbial diseases. However, the resistance may enter at least one of the tested pathogens[16]. Due to the increase in the outbreak of bacterial diseases, the development of bacteria resistance, new antibacterial agents are required [17].The normal intestinal *enterococcus* resist colonization by extraneous organisms. The stability of the normal flora was upset by improper usage of antibiotics. The third generation antibiotic cephalosporin was used as marker for enterococcal superficial infection. Microorganisms acquire, the resistance towards common antibiotics by altering their metabolism and genetic structure[15]. There is evidence that elimination of mucus layer noticible increase in the population of bacteria directly adherence to surface and dissemination to liver and kidney. The present study shows VRE in bacteremia directly translocated to organs and it was treated by ACT 24 solvent extraction which drastically reduced the number of organisms in the different tissues.

CONCLUSION

The finding of this study indicates that exposure of VRE inducing blood bacteremia in Swiss albino rat model developed changes in the organs of the animal. The actinomycetes solvent extract has the ability to reduce VRE in Swiss albino rat model. This indicates the solvent extract of ACT 24 is potential to control VRE and safe or practically non-toxic when orally administered. In future, this research is offering an outset to continue the research by administering the ACT 24 through different routes in different animals species.

CONFLICT OF INTEREST

Conflict of interest declared none.

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