Research Paper

The Sciences 1 (6): 368-371
November- December, 2001

Toxicological Studies of Two Novel Compounds Isolated from *Streptomyces* Species

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The chloroform extract and the two novel compounds streptmysone-A (I) and streptomysone-B (II) of the Czapek-Dox (alkaline) culture filtrate of the *Streptomyces* species were studied for cytotoxic and acute toxicity activities. The cytotoxic activity of the compounds and the extract was determined by brine shrimp lethality bioassay while the acute toxicity study was performed on Swiss albino mice. The LC_{50} values of chloroform extract($CHCl_3$), compound (I) and (II), and standard ampicillin trihydrate were found to be 3.89, 6.31, 125.8 and 6.31μg ml^{-1}, respectively. The LD_{50} values were found to be 66.34 and 79.49mg kg^{-1} for the compound (1) and (II), respectively.

Key words: *Streptomyces*, streptomysone-A, streptomysone-B, toxicity

The Sciences (ISSN 1608-8689) is an International Journal serving the International community of Medical Scientists

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Introduction

In recent years, attempts have been made to investigate the indigenous drugs of choice in infectious diseases for mitigation of suffering of the vast masses of humanity. Scientific development of the research field is a significant aspect to have safer antimicrobial principle through isolation, characterization and biological screening. The first systematic search for antibiotics, made by Gratia and Dath around 1924 (Henka and Dietz, 1940), resulted in the discovery of actinomycetin in strains of *Actinomycetes,* soil organisms that are representative of the group that has given us a number of antibiotics since 1940. The *Actinomycetes* and in particular the genus *Streptomyces* have been identified as one of the most potential sources of antibiotics which are used therapeutically e.g., streptomycin (*Streptomyces grius* 1944), chloramphenicol (*Streptomyces venezuelae* 1947), chlorotetracycline (*Streptomyces aureofaciens 1948)*, mitomycin (*Streptomyces caespitous* 1958) etc.

Keeping these in view, tried to find out new organisms with, antimicrobial property and a strain of *Streptomyces* was isolated from the soil of Pabna, Bangladesh. Two novel compounds were separated from the extract of the culture filtrate of *Streptomyces* species and were identified as streptomysone-A (I) and streptomysone-B (II). The elucidation of structure and antimicrobial screening of these two compounds (Anisuzzaman, 2000) were conducted. The toxicological studies of new antimicrobial compounds are always useful for the development of safer chemicals and for rational treatment of the manifestations of toxicity (Goldstein *et al.*, 1986). We herein, report the cytotoxicity and acute toxicity of both the compounds.

Materials and Methods

Collection of organism: The organism was isolated from a soil sample collected from Pabna, Bangladesh at the depth of 0.75m during the month of October, 1999 using "crowded plate technique" (Hammond and Lambert, 1978). The organism was identified as *Streptomyces* species (Holt *et al.*, 1994) by morphological and biochemical study.

Isolation and characterization of the compounds: The compounds used were obtained from the *Streptomyces* species and were isolated from the culture filtrates by extraction with chloroform ($CHCl_3$), the extract on thin layer chromatographic (Egon and Stahl, 1969) resolution yielded two antimicrobial agents isolated. These were identified as streptomysone-A (I) and streptomysone-B (II) (Fig. 1) on the basis of their UV, IR, NMR and Mass data (Anisuzzaman, 2000).

Determination of cytotoxic activity: The cytotoxic activity of the $CHCl_3$ extract and two isolated compounds (I) and (II) were determined by brine shrimp lethality bioassay (Mayer *et al.*, 1982; Mclaughin and Anderson, 1988; Mclaughin, 1990).

Studies of Acute Toxicity: During screening of a new drug, acute toxicity is done to estimate the nature and extent of acute toxicity and serious abrupt side effects that may follow the administration of the drug.

Determination of LD_{50}: LD_{50} is the dose that is likely to cause the death of 50% of the test animal. It is the most common measure of acute toxicity. In the LD_{50} determination each animal is classified as dead or alive at specified time after drug administration. LD_{50} of the compounds were calculated by usual procedure (Gilman *et al.*, 1980).

Collection of experimental mice: Thirty male Swiss albino mice having age 7 weeks were collected from international Center For Diarrhoel Diseases Research, Bangladesh (ICDDR, B).

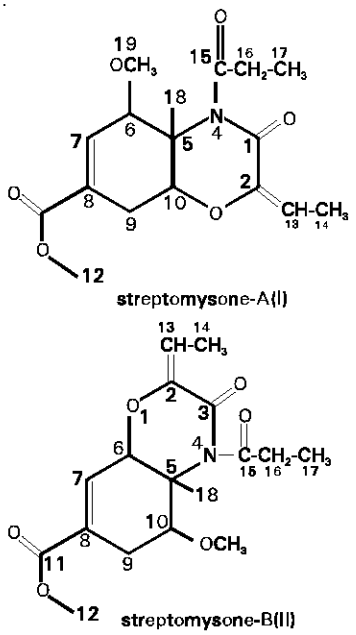

Fig. 1: Structure of streptomysone-A (I) and streptomysone-B (II).

Maintenance of the mice: The mice were housed and caged individually with proper marking. They were kept in clean room with an optimal room temperature. The animals were maintained on standard balanced diet for 15 days prior to administration and continued until completion of the experiment.

Grouping of the mice: Mice were weighed individually and divided into six groups. Each group comprises of 5 mice. Group A received the vehicle only to act as control, while the other groups received the compounds.

Preparation of compounds solution and administration: Compound (I) and (II) (75mg, each) was dissolved separately in 7.5 ml distilled water with the help of Tween 20 to give the final concentration of 10mg ml^{-1}. The Compounds were administered intraperitoneally to each of the experimental mice according to the experimental schedule.

Results and Discussion

Cytotoxicity of the Compound (I) and (II): The results of the brine shrimp lethality bioassay were shown in Table 1. Test sample showed different morality rate at different concentration. The mortality rate of brine shrimp nauplii was found to be increased with the increase of concentration of the samples. A plot of logarithm of concentration versus percent mortality (Goldstein, 1974) was plotted and a best-fitted line was drawn which showed an almost linear

Table 1: Results of brine shrimp lethality bioassay

Group	Concentration of chloroform ($CHCl_3$)	Log C	No. of Shrimp	No of death in each vial			Average no. of death	% Mortality	LC_{50} from the graph ($\mu g\ ml^{-1}$)
				Vial	Vial	Vial 3			
Chloroform extract	5	0.69	10	5	5	6	53.33	53.33	
	10	1.0	10	6	7	7	6.666	66.66	
	20	1.3	10	7	7	8	7.333	73.33	3.89
	40	1.6	10	8	9	9	8.666	86.66	
	80	1.9	10	9	10	10	9.666	96.66	
Ampicillin trihdrate	5	0.69	10	5	4	5	4.666	46.66	
	10	1.0	10	5	5	6	5.333	53.33	
	20	1.3	10	6	7	7	6.666	66.66	6.31
	40	1.6	10	8	8	7	7.666	76.66	
	80	1.9	10	8	9	7	8.000	80.00	
Control	20μl DMSO	0	10	0	0	0	0	0	
Compound (I)	5	0.69	10	4	4	4	4.0	40.00	
	10	1.0	10	4	5	5	4.666	46.66	
	20	1.3	10	6	5	6	5.666	56.66	125.8
	40	1.6	10	7	6	5	6.0	60.00	
	80	1.9	10	7	7	6	6.666	66.66	
Compound (II)	5	0.69	10	5	5	6	5.333	53.33	
	10	1.0	10	6	6	6	6.0	60.00	
	20	1.3	10	6	7	7	6.666	66.66	6.31
	40	1.6	10	8	8	8	8.0	80.00	
	80	1.9	10	8	9	8	8.333	83.00	

Table 2: Acute toxicity data of compound (I)

Group	Dose (mg Kg^{-1})	Log (Dose)	Probit unit	LD_{50} from graph (mg)
A	Vehicle only	-	-	
B	25	1.39	4.48	
C	50	1.69	4.48	66.34
D	100	2.0	5.4	
E	200	2.301	8.97	

Table 3: Acute toxicity data of compound (II)

Group	Dose (mg Kg^{-1})	Log (Dose)	Probit unit	LD_{50} from graph (mg)
A	Vehicle only	-	-	
B	25	1.39	4.16	
C	50	1.69	4.97	79.49
D	100	2.0	5.84	
E	200	2.301	8.97	

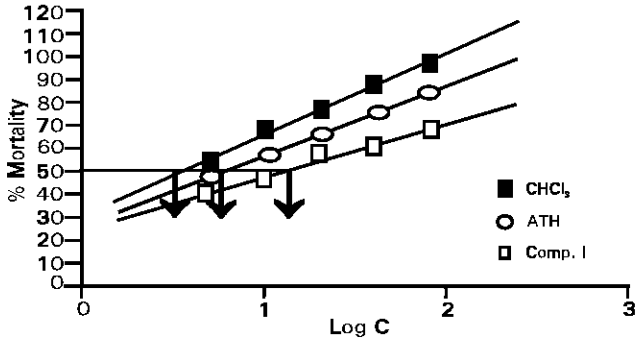

Fig. 2: Brine shrimp lethality bioassay of chloroform extract ($CHCl_3$) ampicillin trihydrate (ATH) and compound (I)

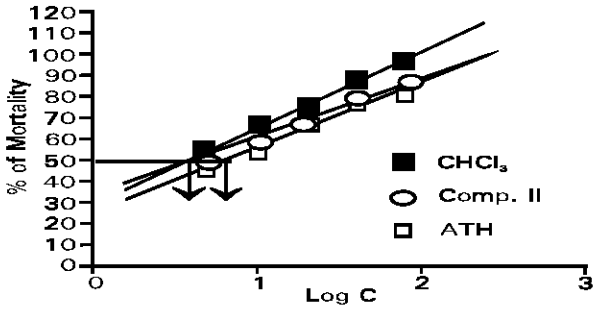

Fig. 3: Brine shrimp lethality bioassay of chloroform extract ($CHCl_3$) ampicillin trihydrate (ATH) and compound (II)

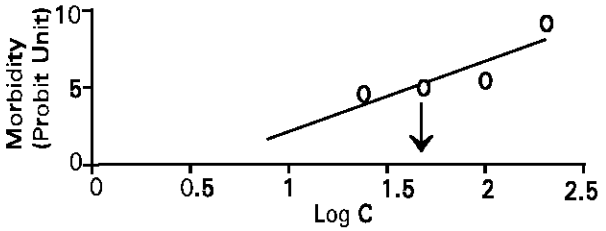

Fig. 4: LD_{50} of the compound (I)

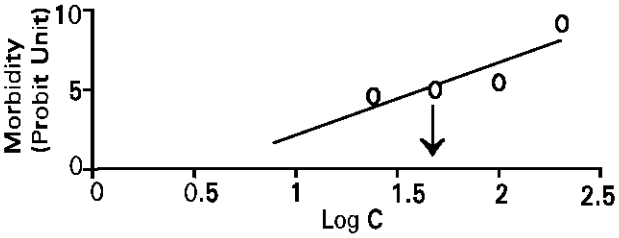

Fig. 5: LD_{50} of the compound (II)

correlation. The median lethal concentration (LC_{50}) was calculated by extrapolation from the graph (Fig. 2 and 3). The LC_{50} values of $CHCl_3$ extract, compound (I) and (II), and standard sample ampicillin trihydrate (ATH) were found to be 3.89, 6.31, 125.8 and 6.31μg ml^{-1}, respectively.

So, it was evident that the extract was lethal to the brine shrimp nauplii as well as biologically active. The compound (II) was less cytotoxic with higher LC_{50} value than that of the other test sample. The extract was comparatively more active with lower LC_{50} values than the compounds, which were found with higher LC_{50} value (Anissuzzaman *et al.*, 2000). The increased activity of the extract may explain the presence of any synergistic compound (s) in the crude extract other than the isolated compounds.

Acute toxicity studies: After the administration of the drug to the mice, both experimental and control groups were observed strictly during the 14 days of experimental period. The relevant signs were recorded including body weight, behavior, CNS excitation, muscle weakness, salivation, diarrhea, food intake, depression etc.

The LD_{50} were determined graphically (Fig. 4 and 5). The logarithms of dosage regimen were plotted in X-axis and the mortality rates in probit units (Goldstein *et al.*,1974) were plotted in Y-axis, these gave straight lines. Then LD_{50} was determined by drawing a vertical line on the X-axis from the point of the straight line where the probit unit 5 (50% mortality) intercepted. The LD_{50} values (Table 2 and 3) were found to be 66.34 and 79.49mg kg^{-1} body weight for the compound (I) and (II), respectively when administered intraperitoneally. From the value of $LD_{50,}$ it can be concluded that the drugs can be used at higher doses.

There was no mortality in the control group. So, it was concluded that both the compounds were biologically active.

Acknowledgments

The authors would like to thanks to Dr. Anwarul Habib, Lecturer, Department of Pharmacology, Rajshahi Medical Collage, Rajshahi, Bangladesh for helping during toxicological studies.

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MS received 29th August, 2001; accepted 2nd November, 2001