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Toxicological Studies of an Antimicrobial Compound and Ethyl Acetate Extract from *Streptomyces bangladeshiensis* sp. nov., on Long Evan's Rats

[1]M. Abdul Alim Al-Bari, [2]M. Abu Sayeed, [3]M.M. Sazedur Rahman and [1]M. Anwar Ul Islam
[1]Pharmaceutical Microbiology Research Laboratories, Department of Pharmacy,
University of Rajshahi, Rajshahi 6205, Bangladesh
[2]Department of Pharmacy, University of Southeast, Banani, Dhaka, Bangladesh
[3]Department of Biochemistry, Islami Bank Medical College, Rajshahi, Bangladesh

Abstract: The toxicological studies of an antimicrobial compound and ethyl acetate extract isolated from a culture broth of a new strain of *Streptomyces bangladeshiensis* (collected from soil) were performed on long Evan's rats. The studies included monitoring the gross general observation such as changes of body weight, checking hematological profiles and biochemical parameters of blood such as SGOT, SGPT, serum alkaline phosphatase, creatinine, uric acid, urea and the histopathology of the liver, kidney, heart, lung, spleen. The results of hematological and biochemical tests showed that the compound AA-2 and the extract did not cause any considerable changes in the values of the parameters observed in control group rats.

Key words: *Streptomyces bangladeshiensis*, sub-acute toxicity, SGOT and SGPT

INTRODUCTION

Pharmaceutical microbiology research provides a basis for the development of new approaches to combat human diseases[1]. Infectious diseases are leading health problems with high morbidity and mortality in the developing countries[2,3]. On this viewpoint, 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 compounds through isolation, characterization and biological screening. The first systemic search for antibiotics, made by Gratia and Dath around 1924, resulted in the discovery of actinomycetin in strain of Actinomycetes, soil organisms that are representatives of the group that has given us a number of antibiotics since 1940. The Actinomycetes and in particular the genus *Streptomyces* is widely reported for the production of various antibiotics which are used therapeutically[4-6]. On this background attempts are taken to find out new organisms with antibiotic property. With this objective in view, a new strain of *Streptomyces bangladeshiensis* was isolated from local soil and then identified[7]. The toxicological studies of new antimicrobial compounds are useful for the development of safer chemicals and for rational treatment of the manifestations of toxicity[8,9]. In this investigation, the toxicological studies were performed with the isolated compounds, ethyl acetate extract and bis- (2-ethylhexyl) phthalate.

MATERIALS AND METHODS

Collection of organism: The organism was isolated from a soil sample collected from Natore, Bangladesh at the depth of 0.75 m during September-October 2001 using crowded plate technique[10]. The organism was identified as novel Actinomycetes, Streptomyces bangladeshiensis on the basis of morphological, physiological, biochemical and sequencing of 16S rDNA studies[7]. The strain has been deposited under the accession number NRRL B-24326 ^{T} (ARS Culture Collection, Peoria, Illinois) and LMG 22738 ^{T} (BCCM /LMG Bacteria Collection).

Extraction, isolation and characterization of the compounds: The maximum secretion of metabolites from the strain was found at the 7th day of incubation in modified Czapek Dox broth (alkaline pH 8.5) medium at 32.5°C by maintaining all the physicochemical factors in optimum level for the culture[11]. The ethyl acetate extract was subjected to column chromatography on column-graded silica gel with gradient elusion using chloroform-ethyl acetate mixtures. The compound AA-2 was purified on preparative-TLC (applied on silica gel 60, $PF_{254+366}$

Corresponding Author: M. Abdul Alim Al-Bari, Pharmaceutical Microbiology Research Laboratories, Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh

MERCK; glass plates 20 and 20 mm, 0.25 and 0.5 mm MERCK) using chloroform-ethyl acetate (5:1) as eluent and UV light (254 and 366 nm) was used for detection and identified as bis- (2-ethylhexyl) phthalate on the basis of its spectral data.

Collection of test animal: For the purpose of sub-acute toxicity studies, nine male Long Evan's rats (age 7 weeks) were collected from the Animal Resources Branch of International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) Mohakhali, Dhaka. Individual weight of rats was taken and they were divided into three groups (A, B and C), each comprising of four rats. The rats were kept in properly numbered iron cages and were supplied with basal diet. The rats were maintained in this way for 14 days before drug administration and continued up to the end of the experiment.

Preparation and administration of compound AA-2 and ethyl acetate extract: The compound AA-2 and ethyl acetate extract were dissolved separately in distilled water with the help of Tween-20 so that 0.2 mL contained 300 μg of these compounds and administered intraperitoneally at a dose of 300 μg/rat/day for 14 consecutive days to each of the experimental rats of group B and group C according to the experimental schedule. Rats of group A received only vehicle and served as a control.

Gross general observation: The body weight of each rat of both groups was taken before the administration of the compound and just prior to sacrifice them. During the whole experimental period, their behavior, Central Nervous System (CNS) excitation, CNS depression, food intake, salivation, diarrhea, muscular weakness, reflexes and urination were monitored.

Hematological profiles of blood: The hematological studies, blood was withdrawn from the tail veins of all the rats in the individual groups before the administration of the compound, at the 7th day and after the rats were sacrificed at the end of the experiment. Then blood smears were made on glass slides and stained with 'Leishmen reagents to estimate Total Count (TC), Differential Count (DC), platelet count and Erythrocytes Sedimentation Rate (ESR). With the use of capillary tubes blood was drawn from each of the rats to estimate the hemoglobin percentage by a hemocytometer.

Biochemical parameters of blood: For the biochemical study, blood were collected from the throat vein of each of the rats after sacrificed them at the end of 14 days of the administration of the compound and determined Serum Glutamate-Oxalo-acetate Transaminase (SGOT), Serum Glutamate-Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (SALP), Serum Bilirubin, Creatinine, uric acid and urea were determined by using the procedures and reagents described in Boehringer Mannheim GmbH diagnostica[12-15].

Histopathological studies: For histopathological studies of liver, kidney, heart, lung and spleen, all of the rats of both groups were sacrificed at 14th day of treatment and these tissue sample were collected separately, sliced into pieces, fixed in formalin (10%) for three days, processed (dehydrated in ascending order of ethanol and embedded in paraffin) stained with 'Harris Hematoxylin and eosin reagent, mounted on glass slides with diphenylxylene and observed under microscope at the Bangladesh Sericulture Institute, Rajshahi, Bangladesh.

Statistical analysis: Results are presented as the mean±SD. Student's t-test was used for comparison between the experimental and control groups. $p<0.05$ value was considered to be statistically significant.

RESULTS AND DISCUSSION

The structure of the compound whose toxicological studies were performed on rats in order to assess the safety of the compound is shown in Fig. 1.

Gross general observation: The average and individual body weights of all rats were increased before and after drug administration (Table 1). Weight gains in the experimental animals after 14 days were 1.289% for

Table 1: Effects of ethyl acetate extract and compound AA-2 on the body weight of rats

Group n=4	Dose (μg/rat/day)	Body weight changes (g) Before treatment	After treatment	% Change
A	300	126.449±4.488	127.970±4.337	+1.041
B	300	137.880±6.086	139.867±5.792	+1.289
C	300	139.744±3.700	141.401±3.234	+1.324

n= number of rats, + = increase

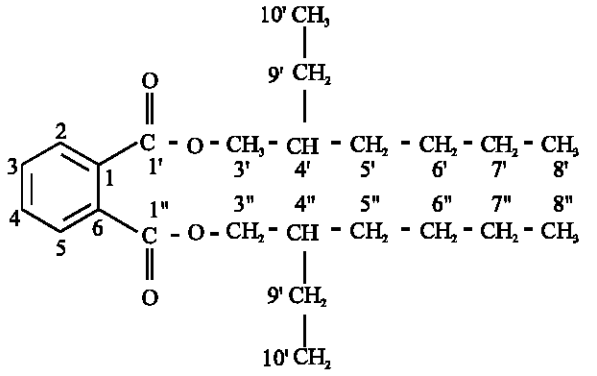

Fig. 1: The structure of compound AA-2(bis-(2-ethylhexyl) phthalate)

Table 2: Effects of compound AA-2 and ethyl extract on hematological profiles rats

Hematological parameters	Group A			Group B			Group C		
	1st day	7th day	14th day	1st day	7th day	14th day	1st day	7th day	14th day
Total RBC count (million/cu mm)	4.633±00.140	5.00±0.143	5.43±0.140	5.233±0.407	4.90±0.083	5.00±0163	5.43±0.292	5.20±0.164	5.000±0.216
Total WBC count (million/cu mm)	12.360±00.180	12.53±0.225	12.25±0.610	11.060±0.233	12.73±0.265	11.43±0.341	11.20±0.889	11.60±0.861	10.730±0.562
Differential count									
Neutrophil	60.670±01.700	59.00±0.816	60.30±1.700	59.670±2.867	60.00±1.632	57.67±2.630	61.60±2.060	57.30±1.250	56.330±2.060
Lymphocyte	37.670±01.247	36.67±0.942	35.33±1.240	38.330±2.054	35.00±1.632	39.00±2.160	37.00±2.160	39.30±1.247	40.330±1.247
Monocyte	0.330±00.475	1.33±0.471	1.00±0.816	0.670±0.471	1.00±0.000	1.33±0.471	0.33±0.471	1.00±0.000	0.330±0.471
Eosinophil	1.330±00.471	2.67±0.471	3.00±0.816	1.330±0.471	3.00±0.000	1.67±0942	1.00±0.000	2.33±0.047	2.3303±0.472
Platelet count (No./cu mm)	308333.000±11.790	316666.00±5.142	336666.00±12.650	283333.000±5.656	2916660.00±0.653	303333.00±2.721	353333.00±0.522	342333.00±1.770	335333.000±0.592
Hemoglobin (%)	54.670±0.542	55.67±0.544	56.00±0.816	60.000±3.741	61.33±0.471	63.33±1.247	58.30±1.247	60.00±1.414	59.67±1.240
ESR (1st h)	14.000±1.632	15.00±1.414	14.67±0.470	18.600±0.471	18.70±0.471	19.67±0.471	15.60±0.943	16.00±0.816	16.33±0.943

n= number of rats,+ = increase

Table 3: Effects of compound AA-2 and ethyl acetate extract on biochemical parameters of control and experimental rats

Biochemical parameters	Groups			% Change	
	A	B	C	B	C
SGPT (IU/L)	8.333±0.478	8.433±0.499	8.50±0.408	+1.00	+2.000
SGOT (IU/L)	9.330±0.250	9.893±0.499	9.83±0.249	+5.60	+5.300
SALP (IU/L)	36.330±1.250	37.000±2.160	39.33±2.490	+7.70	+7.630
Bilirubin (mg/dL)	0.340±0.016	0.340±0.014	0.36±0.008	+0.20	+5.880
Creatinine (mg/dL)	0.606±0.027	0.631±0.059	0.62±0.010	+5.00	+3.300
Blood urea (mg/dL)	14.000±2.450	15.000±4.083	14.33±0.490	+10.35	+2.340
Uric acid (mg/dL)	7.000±2.450	7.000±0.082	7.03±0.217	+1.36	+0.471

+ = increase

Table 4: Effects of compound AA-2 and ethyl acetate extract on histopathology of rat's organs

Groups	Dose (µg/ rat/day)	Histopathological changes				
		Heart	Kidney	Liver	Lungs	Spleen
A	300	NAD	NAD	NAD	NAD	NAD
B	300	NAD	NAD	NAD	NAD	NAD
C	300	NAD	NAD	NAD	NAD	NAD

NAD: No Abnormality Detected

compound AA-2 and 1.324% for ethyl acetate extract compared 1.041% in control animals. This is because the rats were in the growing stage. Thus body weight increased with time the Group A showed less growth rate than the Group B indicating that the compound AA-2 has got some effect on normal growth of rats. The rats of Group A, B and C showed no signs of tremor, convulsion and reflex abnormalities. No muscular numbness of the hind and forelegs, salivation and diarrhea was observed. The food intake per day was also being found normal. After administration of extract and the compound AA-2, most of the parameters were slightly increased with respect to control rats but remained within the normal range (Table 1).

Hematological profile: The hematological profiles were studied on normal rats after first, 7th and 14th day of treatment. Each time the value of the parameters in each rat were changed slightly. However, the parameters remained within the normal range. The findings of hematological profiles (Table 2) indicates that the parameters of hematology of control and drug treated rats have no detectable differences i.e. they have no substantial effect on hematological structure.

Biochemical parameters of blood: Biochemical parameters were studied in normal rats (before treatment) and after 7 and 14 days of treatment (Table 3). The serum urea concentration was found to be decreased after AA-2 and the extract administration (Group B). The changes in other parameters were within the normal range and statistically insignificant. The reason of low urea concentration might be one of the followings; over hydration, inappropriate secretion of antidiuretic hormone, severe liver disease.

Histopathological studies: Histopathological studies of liver, kidney, lung, heart and spleen of the control and experimental rats were carried out after intraperitoneal administration of the compound AA-2 and ethyl acetate extract for 14 days at a dose 300 µg/rat/day (Table 4). No detectable differences in the histopathology of these organs of control and drug treated rats were observed. This indicates that the compound AA-2 and the extract have no effect on cellular structure i.e. they do not cause degeneration of the cells of these organs.

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