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Sub-Acute Toxicity Study of an Antimicrobial Metabolite from *Streptomyces lalonnensis* Sp. Nov., on Long Evan's Rats

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Abstract: The sub-acute toxicity of an antimicrobial metabolite (*E*)-1-(3-hydroxyazetidin-1-yl) undec-7-ene-1,5-dione isolated from a new *Streptomyces* species on Long Evan's male rats was studied. This investigation was designed to check gross general observations, hematological profiles, biochemical parameters of blood and histopathology of the liver, kidney, lung, spleen and heart tissues of both experimental and control rats. The body weight was increased after completion of drug treatment. However, the metabolite showed no detectable abnormalities on hematological profiles, biochemical parameters of blood and histopathological investigations of experimental rats at a dose of 300µg/rat/day for 14 consecutive days in comparison to those produced by control group.

Key words: *Streptomyces lalonnensis* · (*E*)-1-(3-hydroxyazetidin-1-yl) undec-7-ene-1,5-dione · Hematological profile · Histopathology

INTRODUCTION

The concept of antibiosis opened a new field of research for isolation of antibiotics from microorganism and so far more than 4000 such antibiotics are known. Soil bacteria and fungi have played a significant role in antibiotic discovery. 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]. The first systemic search for antibiotics, made by Gratia and Dath around 1924 [4] resulted in the discovery of actinomycetin from a strain of Actinomycetes, a group of soil organisms that has given us a number of antibiotics since 1940. Each actinomycete strain probably has genetic potential for producing 10-20 secondary metabolites [5]. The Actinomycetes especially the genus *Streptomyces* is widely reported for the production of bioactive compounds, notably antibiotics, enzyme inhibitors and pharmacologically active agents [6-8]. As a result, secondary metabolites produced by *Streptomyces* have been the primary source of antibiotics and more recently, are used as herbicides, anticancer drugs, immunoregulators and antiparasitic compounds [9-12].

As part of our ongoing research on microbial metabolites [13,14] attempts were taken to find out new organisms with antimicrobial properties. With this concept, a strain of *Streptomyces* was isolated from a soil sample of Kustia, Bangladesh with antimicrobial principle and identified as a new species. From the pet. ether extract of the culture of this organism, a new antimicrobial compound was isolated and identified as (*E*)-1-(3-hydroxyazetidin-1-yl) undec-7-ene-1,5-dione (compound 1). Pharmacology is toxicology at higher doses. Even at therapeutic doses many drugs show unavoidable toxic effects. Therefore, for the assessment of a drug it is necessary to study toxicity in animals like rat, guinea pigs, dogs, monkey etc. under various conditions of drug administration. This led to the present investigation on the sub-acute toxicity of the isolated compound at a dose of 300µg/day/rat on long Evan's rats for 14 consecutive days.

MATERIALS AND METHODS

Collection and Identification of Organism: The organism was isolated from a soil sample collected from Kushtia, Bangladesh at the depth of 0.75m using crowded plate technique [15]. The 16S rDNA sequence generated in

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this work was compared with the 16S rDNA sequences of other organisms retrieved from the EMBL/ GenBank database. The nearest is being *Streptomyces parpurascens*, with 99.3% nucleotide similarity. In addition, the morphological, physiological and biochemical properties of the present isolate was also different from *Streptomyces parpurascens* [16]. On the basis of morphological, physiological, biochemical and sequencing of 16S rDNA studies, the organism was identified as a novel *Streptomyces* species and named as *Streptomyces lalonnensis* [16].

Extraction, Isolation and Characterization of the Compounds: The maximum secretion of metabolites from the strain was found at the 10th day of incubation in modified Czapek Dox broth (alkaline pH 8) medium at 37.5°C by maintaining all the physicochemical factors in optimum level for the culture [16]. The pet ether extract was subjected to column chromatography on column-graded silica gel with gradient elusion using pet. ether-ethyl acetate mixtures. The compound 1 was purified on preparative-TLC (applied on silica gel 60, $PF_{-254+366}$ MERCK; glass plates 20 and 20 mm, 0.25 and 0.5 mm MERCK) using pet. ether:chloroform (10:1) as eluent and UV light (254 and 366 nm) was used for detection and identified on the basis of its spectral data.

Collection of Test Animal: For the purpose of sub-acute toxicity studies, eight 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 two groups (G-1 and G-2), 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 Sample: The compound 1 was dissolved in distilled water with the help of Tween-20 (i.e. 0.2 ml contained 300 µg of metabolite) and administered intraperitoneally at a dose of 300 µg/rat/day for 14 consecutive days to each of the experimental rats of group G-2 according to the experimental schedule. Group G-1 rats 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 1 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: For the hematological studies, blood was withdrawn from the tail veins of all the rats in the individual groups before the administration of the compound 1, 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 reagent' to estimate Total Count (TC) of RBC and WBC, Differential Count (DC) of WBC and platelet count. Blood was also drawn from each rat [17] with the help of capillary tube for estimating the haemoglobin percentage by Van Kampen-Ziftra's method. The test was repeated on 7^{th} and 14^{th} day [17] after administration of the compound 1.

Biochemical Parameters of Blood: For the biochemical study, blood was 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 1 and SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase), serum bilirubin, creatinine and urea by using the usual procedures and reagents as described in Enlehringer Mannheim GmbH Diagnostica [18-21].

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 diphenyl xylene 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

Column chromatography followed by preparative TLC of the pet. ether extract yielded compound 1. Compound 1 was identified as (*E*)-1-(3-hydroxyazetidin-1-yl) undec-7-ene-1,5-dione (Figure 1). To our best knowledge this is a new compound.

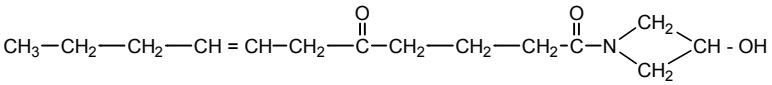

Fig. 1: Structure of compound 1

Table 1: Effect of compound 1 on the body weights of rats after intraperitoneal administration

Group	Dose µg/rat/day	Body weight before drug treatment (gm), n=4 $M_1 \pm SD_1$	Body weight After drug treatment (gm), n=4 $M_2 \pm SD_2$	% Change	Calculated 't' values	't' values at 5% level of significance	Remark
G-1	300	151.12±1.4	154.62±1.1	+2.32	+3.38	2.447	NS
G-2	300	158±3.88	170.5±3.6	+7.83	+4.67	2.447	NS

M_1 and M_2 = Mean value; SD_1 and SD_2 = Standard deviations and NS = Not significant

Table 2: Effects of compound 1 on hematological profile of rats

	Group-1			Group-2		
Hematological Parameter	1st day (M±SD)	2nd day (M±SD)	3rd day (M±SD)	1st day (M±SD)	2nd day (M±SD)	3rd day (M±SD)
Total RBC count (million/mm^3)	5.11±0.01	5.12±0.017	5.21±0.025	5.57±0.07	5.55±0.07	5.55±0.27
Total WBC count (no./mm^3)	5050±50	4948±48	5150±72	6135±72	5994±82	5985±112
Differential count (no./mm^3)						
Neutrophil	60.7±1.70	59±0.16	60.3±0.70	59.7±2.87	60±1.32	57.7 ± 2.3
Lymphocyte	37.68±1.2	36.68±0.94	35.34±1.25	38.33±2.0	35.11±1.6	39.12 ±2.16
Monocyte	0.32±0.47	1.32±0.47	1.3±0.815	0.66±0.47	1±0	1.32 ±0.47
Eosinophil	1.33±0.47	2.67±0.471	3.0±0.816	1.33±0.47	3 ± 0	1.67 ± 0.94
Platelet count (no/mm^3)	308366±11.7	316333±5.1	336633±12.6	283344±5.6	291655±0.6	303335±2.7
Hemoglobin (%)	54.88 ±0.54	55.77±0.54	56.1±0.81	60±3.75	61.44±0.48	63.3±1.24
ESR (1st hour)	14±1.0	14±2.3	14±5.1	19±0.0	19±0.01	19±0.2

Table 3: Effect of compound 1 on biochemical parameters on rats

Biochemical parameter	Control rats (G-1), n=4 $M_1 \pm SD_1$	Experimental rats, (G-2), n=4 $M_2 \pm SD_2$	% Change	Calculated 't' values at 5% level of significance	't' values	Remark
SGPT (IU/L)	10.75±1.92	11.5±1.12	+6.97	+.674	2.447	NS
SGOT (IU/L)	8.75±1.3	12.25±1.48	+40	+3.66	2.447	NS
SALP (IU/L)	38.75±3.56	33±1.87	-14.83	-2.85	2.447	NS
Creatinine (mg/dl)	1.065±0.19	1.12±1.41	+3.75	+0.30	2.447	NS
Uric acid (mg/dl)	7.025±0.45	6.8±0.47	-3.20	-0.69	2.447	NS
Urea (mg/dl)	38.75±4.76	44±3.93	+13.54	+1.69	2..447	NS

M_1 and M_2 = Sample mean value; SD_1 and SD_2 = Standard deviation; n = Number of rats; + = Increases; - = Decreases; NS = Not significant.

Table 4: Effect of compound 1 on histopathology of rat's organs

		Histopathological changes				
Group	Dose (µg/ rat/day)	Heart	Kidney	Liver	Lungs	Spleen
G-1	300	NAD	NAD	NAD	NAD	NAD
G-2	300	NAD	NAD	NAD	NAD	NAD

NAD = indicates no abnormality detected

Gross General Observation: The rats of group A and B were treated with vehicle and compound 1 respectively exhibited no signs of tremor, convulsions 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. The average and individual body weights of all rats were increased after antimicrobial metabolites administration which was statistically insignificant (Table 1). From these observations we can conclude that the isolated compound has no adverse effect on normal growth of long Evan's rats.

Hematological Profiles: The hematological profiles were studied on normal rats after 1st, 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 metabolite 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) to detect any sort of pathological changes of liver and kidney. The results shown in Table 3 indicated that the biochemical parameters changed slightly and remained within the normal range. So it may be concluded that the compound has no untoward effect on both liver and kidney function of the experimental rats.

Histopathological Studies: Histopathological studies of liver, kidney, lung, heart and spleen of the control and experimental rats were carried out after intraperitoneal administration of vehicle and the compound 1 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 1 has no effect on cellular structure i.e. they do not cause degeneration of the cells of these organs.

CONCLUSION

In recent years, many researches have focused on sub acute toxicity study on antimicrobial metabolites isolated from different *Streptomyces* species [22-24] to establish new drug from this renowned source of bioactive materials. The present study was also designed in similar way to observe the adverse effect of a new compound from a new soil *Streptomyces* species. The findings of present study demonstrate that the compound 1 possess no adverse effect on Long Evan's rats at a dose of 300 µg/rat/day. Thus the findings of this investigation would give valuable support to make further study and clinical trial of the isolated compound.

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Normal Range of Biochemical Parameters: SGOT and SGPT = Normal up to 12 U/L; S. urea = 25-50 µg/dl; SALP = Normal up to 48 U/L; S. creatnine = 0.8-1.5 µg/dl; S.uric acid = 4.2-7.5 µg/dl.

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