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TOXICOLOGICAL STUDIES OF ANTIBIOTICS FROM A *STRPTOMYCES* STRAIN ON LONG EVAN'S RATS

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Abstract: The sub-acute toxicity studies of two antibiotics (S_1 & S_2), isolated from the culture filtrate of a *Streptomyces* strain were carried out on long evan's rats. The study included the gross general observation, body weight changes, hematological profiles, such as, total count of RBC & WBC, differential count of WBC, platelet count & hemoglobin percentage, biochemical parameters of blood, such as, SGOT, SGPT, serum alkaline phosphate, serum bilirubin, serum uric acid & serum urea, and histopathological studies of liver, kidney, heart, lungs & spleen tissues. The antibiotics did not show any abnormalities on the hematological profiles. The body weight and biochemical parameters of blood were found to be slightly changes with respect to that of control but remained within the normal range and statistically insignificant. No detectable abnormalities were observed in the histopathological studies.

Keywords: Toxicological study, Antibiotic, *Streptomyces*

Introduction

Since the discovery of Penicillin in 1929, thousands of antibiotics have been isolated from microorganisms or synthesized. The widespread and successful use of these medical agents has given humankind the perception that they have had the weapons to conquer any infectious disease. Although a wide variety of microorganisms are kept at bay, the newer and resistant strains of bacteria are alarmingly on the rise. Researchers facing with this tremendous microbial challenge, have been searching for newer and more potent agents with redoubled efforts. Several thousand different antibiotics have been isolated from fungi such as *Penicillium, Aspergillus, Actinomycetes* and bacteria and the genus *Streptomyces* is already reputed for the production of antibiotics (Waksman *et al.,* 1940; Schatz *et al.,* 1944). But only a few of those antibiotics are suitable and available for treatment because of their range of activity and less toxicity. Moreover, the emergence of new life threatening diseases has shown the dangerous consequence of complacency (London Sch. Hyg. and Trop. Med. News Let. 1994). As a part of our continuing search for microbial metabolites from soil samples, we collected samples from different part of Bangladesh. We isolated a *Streptomyces* strain with antimicrobial principle from the soil of Pabna. This was cultured in starch casein broth medium and two antibiotics (S_1 & S_2) were isolated from the culture filtrate. For the assessment of a new compound as a drug it is necessary to study its toxicity. Therefore, we investigated the sub-acute toxicity of these compounds on long evan's rats by observing body weight changes, hematological profiles, biochemical parameters of blood serum and histopathology of liver, kidney, heart, lungs & spleen tissues.

The aim of these studies was to evaluate the safety margin of these compounds prior to clinical trial. Because at the therapeutic blood level some drugs have unavoidable toxic effects and all drugs are toxic at higher doses.

Materials and Methods

***Production, isolation and purification of the antibiotics*:** The *Streptomyces* was isolated from a soil sample collected from Pabna, Bangladesh at a depth of 0.5 meter during August 1995 using Crowded Plate Technique (Masud 1997). The *Streptomyces* was cultured in starch casein broth medium at pH 8 and after 21 days of incubation at $38^0C \pm 0.5\ ^0C$, the culture filtrate was extracted with ethyl acetate. The yellowish residue was subjected to thin layer chromatography (TLC) (Marston *et al.*, 1991) using silica gel PF_{254} . Two TLC fractions (S_1 & S_2) were isolated using solvent systems chloroform : methanol (8 : 2). S_1 & S_2 were further purified by thin layer chromatography (TLC) (Marston *et al.* 1991) and preparatory thin layer chromatography (PTLC) over silica gel PF_{254} to yield two antibiotics namely S_1 & S_2 respectively. The purity of the fractions were checked by HPLC using C_{18} bonded silica column (0.46 X 250 mm) and methanol:water (70:30) as the mobile phase for S_1 & methanol:water (60:40) for S_2.

***Collection, grouping and maintenance of the rats*:** Twenty long evan's adult male rats of same age (6 weeks) were collected from the Animal Resources Branch of International Center for Diarrhoeal Disease Research,

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Bangladesh (ICDDR'B). Individual weights of the rats were recorded and they were grouped into A, B, & C, each comprising five rats. The rats of group B & C were used for experimental while that of group A were used as control. The rats were kept in properly numbered iron cages in clean animal houses at room temperature. They were supplied ideal diet (Hawk and Summerson, 1954). The rats were maintained in this way for 15 days before drug administration and continued upto the end of the experiments.

Sample preparation and administration: The antibiotics S_1 & S_2 were dissolved in water with the help of tween 20 and administered intraperitoneal at a dose of 200 µg/rat/day for 21 consecutive days to the rats of group of B & C respectively. Rats of group A received vehicle (water with tween 20) only.

Monitoring the gross general observation, the change in body weights, hematological profiles and biochemical parameters of blood: Before administration the drug, each rat has been weighed individually and these weights were compared with those measured after the administration of the drug *i. e.* prior to sacrificing the animal after 21 days. During the whole experimental period, their behavior, CNS excitement, CNS depression, reflexes of muscular weakness, salivation, urination, diarrhoea etc. were also monitored. For hematological study, blood was drawn from the tail veins of all rats of group A, B & C before drug administration. Blood smears were made on glass slides and stained with Leishen reagent to perform total count (TC) of RBC & WBC, differential count (DC) of WBC and platelet count. With the use of capillary tubes blood was drawn from each rat to estimate the hemoglobin percentage (Hb %) by a Van Kampen-Zijlstra's method. The tests were repeated on the 7th, 14th and 21st day after the commencement of drug administration. For the determination of serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), serum alkaline phosphate (SALP), serum level of bilirubin, creatinine, uric acid and urea, blood samples were collected separately from each of the control and experimental rats from their throat veins after sacrificing them at the end of 21st day of drug administration. The samples were then analyzed for biochemical parameters using the procedures and reagents described in Boehringer Mannheim GmbH Diagnostica (King and Armstrong, 1934; Reitman and Frankel, 1957; Fawcett and Scott, 1960; Coulombe and Favreau, 1963).

Statistical analysis: The results were represented as the mean ±SEM from n experiments. A t-test was used to compare mean values between treatment and control group in the *in vivo* studies. The criterion for a statistically significant difference was $P<0.05$.

Histopathological studies: Histopathological studies of the liver, kidney, heart, lung & spleen tissues were performed to observe any changes in the cellular structure of the rats receiving S_1 & S_2 as compared to that of the control rats. The tissue samples of the above organs were collected after sacrificing them at the end of 21 days of drug administration. These tissues were sliced into pieces, fixed in 10 % formaline for 2 days, processed, embedded in paraffin, sliced in microtome, stained with Erchlich's Haematoxylin and eosin reagent, mounted on glass slides with D. P. X. mounting fluid and observed under high power microscope.

Results and Discussion

Gross general observation and body weight of rats: It was observed that till the 21st day of treatment, any rat of group A, B or C showed no sign of tremor, convulsion and reflex abnormalities. Salvation, diarrhoea or muscular numbness of the fore and hind limbs was also absent. The food intake per day was also found normal. The changes in body weights due to treatment with the compounds were found to be statistically insignificant (Table 1).

Hematological Profiles: Hematological profiles like TC of RBC and WBC, DC of WBC, platelet count and Hb% were studied on normal rats before treatment and after 7, 14 & 21 days of treatment. No abnormalities were found in hematological profiles after drug administration (Table 2.1 and 2.2).

Biochemical parameters: Biochemical parameters *e.g.* SGOT, SGPT, SALP, bilirubin, creatinine, uric acid and urea of rat blood serum were determined after administration of antibiotics S_1 and S_2 at a dose level of 200 µg/rat/day for 21 consecutive days. The results are shown in Table 3. All parameters were found to be very slightly changed after drug administration with respect to control but they were within the normal range and statistically insignificant.

Histopathological studies: The animals of both control and experimental groups were sacrificed at the end of 21st day of drug treatment and the organs such as kidney, liver, heart, lungs and spleen were isolated and histopathological examinations were done (Table 4). No detectable difference of the histopathological view was observed between the control and drug treated rats when the slides of the tissues were examined under high power microscope (Fig. 1). This indicates that the antibiotics have no adverse effects on cellular structure.

Table 1: Effects of antibiotics on body weight of rats after intraperitoneal administration of 200 µg/rat/day for 21 consecutive days.

Group n = 5	Dose (rat/day)	Body weight changes in gm		% change	Calculated t value	t value at 5% level of significance	Remarks
		Before treatment (1st day) $M_1 \pm SD_1$	After treatment (21st day) $M_2 \pm SD_2$				
A (Control)	200 µl	159.4±1.74	161.0±1.41	+1.003	1.60	2.306	NS
B (S_1)	200 µg	161.4±2.33	163.2±1.72	+1.110	1.39	2.306	NS
C (S_2)	200 µg	160.8±1.33	162.4±1.02	+0.990	2.13	2.306	NS

M_1 & M_2 = Mean values SD_1 & SD_2 = Standard deviations n = Number of rats + = Increase, - = Decrease NS = Not significant

Table 2.1: Effects of antibiotics on hematological profiles of rats after intraperitoneal administration of 200 µg/rat/day for 21 consecutive days.

Group n = 5	Total RBC count (million/cc)				Total WBC count (no./cc)				Platelet count (no./cc)				Haemoglobin (%)				Remark
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	
A (Control)	5.06	5.12	5.06	5.10	11,320	11,840	11,980	11,880	3,23,200	3,47,600	3,24,000	3,46,000	88.2	88.4	86.8	87.0	NS
B (S_1)	5.22	5.30	5.56	5.16	11,660	11,840	12,060	12,040	3,20,200	3,66,000	3,52,000	3,52,000	86.2	84.8	88.4	88.0	NS
C (S_2)	5.18	5.64	5.54	5.06	10,760	11,420	11,120	11,600	3,58,600	3,61,000	3,47,600	3,54,000	87.8	85.2	84.0	84.6	NS

I = Mean value before treatment (1st day) II = Mean value after treatment (7th day) III = Mean value after treatment (14th day) IV = Mean value after treatment (21st day)
n = Number of rats NS = Not significant

Table 2.2: Effects of antibiotics on hematological profiles of rats after intraperitoneal administration of 200 µg/rat/day for 21 consecutive days.

Group n = 5	Differential count of WBC																Remark
	Neutrophil				Lymphocyte				Monocyte				Eosinophil				
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	
A (Control)	38.8	39.6	38.0	37.4	50.4	50.6	51.8	51.4	4.4	4.4	3.8	4.4	3.6	3.4	3.4	4.2	NS
B (S_1)	35.0	39.0	37.0	36.0	49.2	48.8	51.0	50.2	4.2	4.0	3.8	4.0	3.6	3.6	3.2	4.0	NS
C (S_2)	36.6	37.2	35.4	37.4	49.6	48.8	51.2	49.2	3.8	3.8	4.4	3.8	4.0	3.6	3.6	4.0	NS

I = Mean value before treatment (1st day) II = Mean value after treatment (7th day) III = Mean value after treatment (14th day), IV = Mean value after treatment (21st day)
n = Number of rats NS = Not significant

Table 3: Effects of antibiotics on biochemical parameters of rats blood after intraperitoneal administration of 200 µg/rat/day for 21 consecutive days.

Biochemical parameters	Group A (Control) n = 5 $M_1 \pm SD_1$	Group B (S_1) n = 5 $M_2 \pm SD_2$	Group C (S_2) n = 5 $M_3 \pm SD_3$	% change		Calculated t value		t values at 5% level of significance	Remark
				Group B	Group C	Group B	Group C		
SGOT (U/L)	29.8±2.13	27.4±1.49	27.6±2.35	-4.16	-4.86	1.20	0.856	2.306	NS
SGPT (U/L)	29.6±1.01	30.6±1.02	30.8±1.16	+4.05	+3.37	1.56	1.74	2.306	NS
SALP (U/L)	37.4±1.74	38.6±1.02	38.8±1.83	+3.76	+3.20	1.33	1.24	2.306	NS
Serum bilirubin (mg/dl)	0.52±0.116	0.56±0.048	0.54±0.102	+3.84	+7.69	0.714	1.53	2.306	NS
Serum creatinine (mg/dl)	0.94±0.149	0.88±0.079	1.04±0.102	+10.6	-6.38	0.796	1.25	2.306	NS
Serum uric acid (mg/dl)	6.6±0.275	6.32±0.116	7.16±0.530	+8.48	-4.24	2.10	2.09	2.306	NS
Serum urea (mg/dl)	41.4±2.41	40.0±1.67	39.6±1.62	-4.34	-3.38	1.06	1.39	2.306	NS

M_1, M_2, & M_3 = Mean values SD_1, SD_2, & SD_3 = Standard deviations n = Number of rats + = Increase, - = Decrease NS = Not significant

Table 4: Histopathological studies after treatment with antibiotics at a dose level of 200 µg/rat/day for 21 consecutive days.

Group	Dose (rat/day)	Histopathological changes observed				
		Kidney	Liver	Heart	Lung	Spleen
A (Control)	200 µl	NAD	NAD	NAD	NAD	NAD
B (S_1)	200 µg	NAD	NAD	NAD	NAD	NAD
C (S_2)	200 µg	NAD	NAD	NAD	NAD	NAD

NAD = No abnormality detected

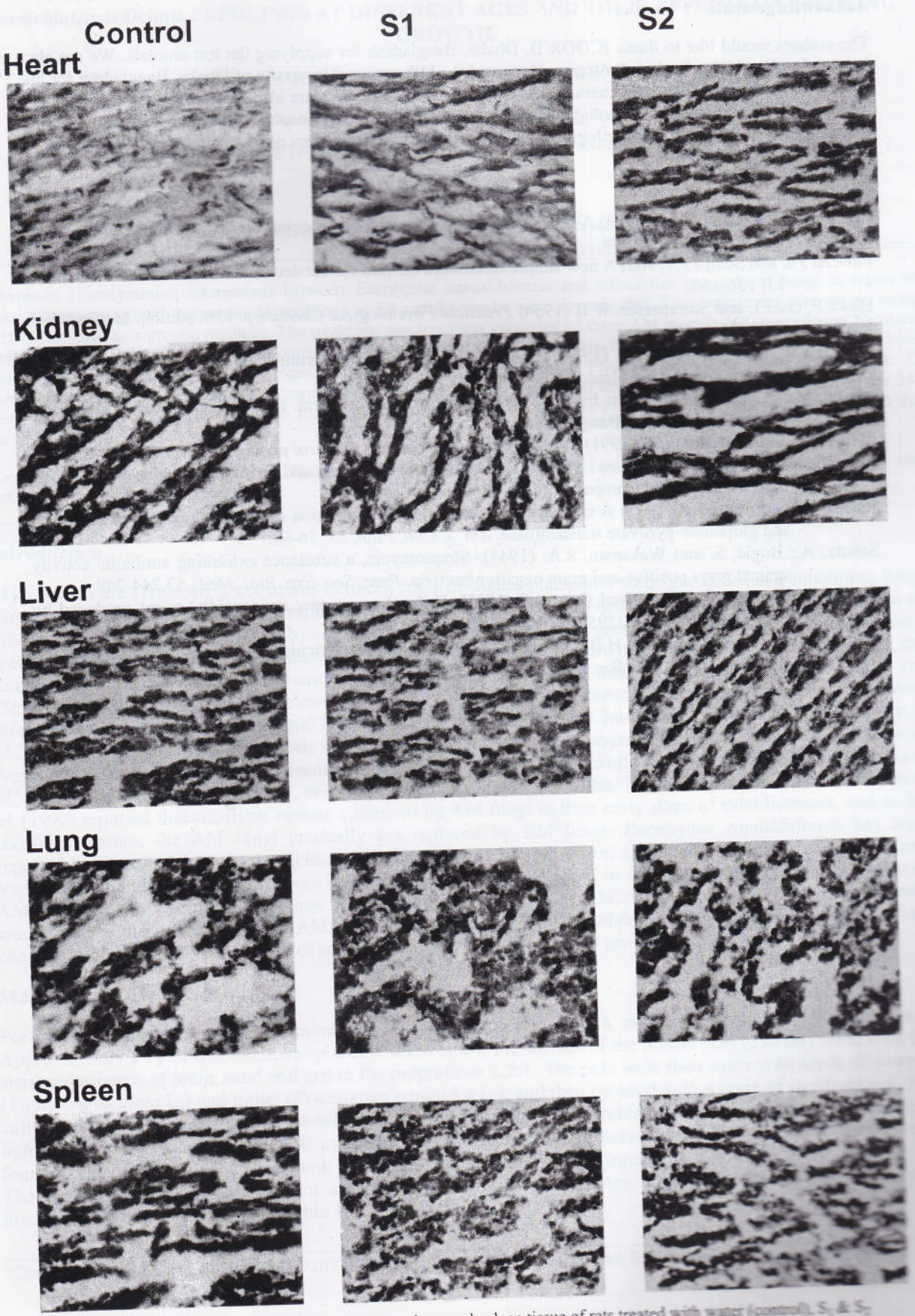

Fig. – 1. Microscopic view of heart, kidney, liver, lung and spleen tissue of rats treated with water (control), S_1 & S_2 respectively after 21 days (X400)

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