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Toxicity Study of 8-Propionato-2-β (D+) Glucosyl-9, 10-Pyranopyridine Isolated from *Streptomyces* species on Long Evan's Rats

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Abstract: The subacute toxicity studies of 8-propionato-2-β (D+) glucosyl-9, 10-pyranopyridine (R-1), isolated from the *Streptomyces* species was carried out in Long Evan's rats. The compound was administered at a dose of 300 µg/rat/day for 14 consecutive days. The gross general observations such as changes of body weight, hematological profiles, biochemical parameters of blood and the histopathology of the liver, kidney, heart and lungs were investigated both in control and experimental rats. The body weights of the rats were slightly increased. The change of hematological and biochemical parameters were statistically nonsignificant. No abnormalities were detected in the histopathology of the liver, kidneys, heart and lungs in the experimental group of rats as compared with control group of rats.

Key words: *Streptomyces* species, 8-propionato-2-β(D+) glucosyl-9, 10-pyranopyridine, subacute toxicity, long Evan's rats

Introduction

Infectious diseases are the leading health problems with high morbidity in developing countries. Among them diarrhoea, acute respiratory tract infections, tuberculosis and recently AIDS are the most serious ones caused by various pathogenic organisms. The antibiotics as well as chemotherapeutic agents are effectively used to combat such pathogenic microorganisms. For this reason, the isolation of variety of antimicrobial agents from microorganisms has been the major contributions of scientific and research community. In recent years, owing to indiscriminate use of antibiotics and other unknown reasons the pathogenic organisms are gaining resistance to the existing antimicrobial agents. Moreover, careless use of antibiotics in many countries causes development of antibiotic resistant strains, which are increasing day by day (Roche, 1985). Hence, the search for a newer, safer and more potent antibiotic against these organisms is a pressing need. Still now the richest source of antibiotics are the *Streptomyces* species (Gennaro, 1990). Therefore, a strain of *Streptomyces* species was collected and identified (Holt, 1994). By using thin layer chromatographic technique (Touchston and Dobbins, 1978), a pure antimicrobial compound (R-1, Fig. 1) was isolated from the chloroform extract and was identified as 8-propionato-2-β (D+)

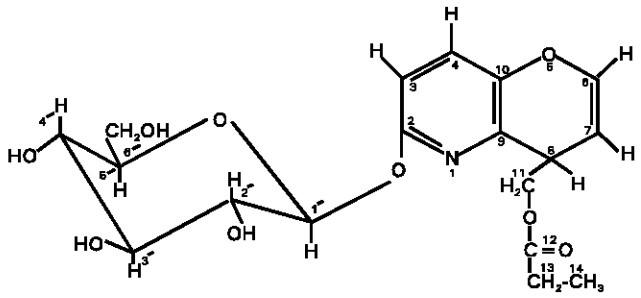

Fig. 1: 8-propionato-2-β (D+) glucosyl-9, 10-pyranopyridine

glucosyl-9, 10-pyranopyridine (Barman, 2002). The antimicrobial screening of the compound was conducted by Barman *et al.* (2002).

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. Toxicological data help to make decision whether a new drug is adopted for clinical use or not. Therefore, in connection of this objective, the present work was conducted to report the toxicological studies of the compound 8-propionato-2-β (D+) glucosyl-9, 10-pyranopyridine in rats.

Materials and Methods

General: The experiment was carried out in Microbiology research laboratory, Department of Pharmacy, Rajshahi University, Bangladesh during March-November, 2001.

Collection of experimental rats: For the purpose of study 16 Long Evan's rats of same sex (male) and age (7 weeks) were collected from the International Center for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B).

Maintenance of the rats: The rats were kept in properly numbered iron cages individually. The diet supplied to each rat was about 20 g^{-1} day, which was approximately isocaloric. They were kept in a clean animal house with an optimal room temperature. The animals were maintained in this way for 15 days before drug administration and continued up to the end of the experiment.

Grouping of rats: Weight of the individual rats was determined and they were grouped into two groups (A, B). Each group contained 4 rats. Group A received vehicle only and acts as control. But the group B received the antimicrobial compound R-1.

Administration of the sample: The compound was dissolved in distilled water with the help of Tween-20 in such a way that 0.3 ml contained 300 µg of the compound. The sample was

administered to the rats of group B intra-peritoneally at a dose of 300 µg/rat/day for 14 consecutive days.

Gross general observation after drug administration: The rats were observed daily very keenly to notify the following features such as behaviour, CNS excitation, CNS depression, food intake, salivation, diarrhoea and muscular weakness.
The body weight of each rat of groups A and B were measured before administration of the drugs and at the completion of the treatment prior to sacrificing the animals. These weights were compared.

Monitoring the haematological profiles, biochemical parameters of blood and histopathology of liver, kidneys, heart and lungs: For hematological studies blood was drawn from the tail veins of all the rats in groups A and B before the commencement of drug administration. Blood smears were made on glass slides and stained with leishmen reagent to perform TC, DC and platelet count. With the use of capillary tubes, blood was drawn from each rat to estimate the haemoglobin percentage by Van Kampen-Zijlstra's method, which is the pre-haematological study on normal rats. Post haematological studies were done on 7th and 14th day after the commencement of drug administration following the same procedure as that done on normal rats.
For the determination of biochemical parameters such as SGOT, SGPT, SALP, serum bilirubin, creatinine and urea, blood samples were collected separately from each of the control and experimental rat from their throat vein after sacrificing at the end of 14th day of compound administration. The samples were then analyzed for biochemical parameters using the procedures and reagents as described in Enlehringer Mannheim GmbH Diagnostica (King and Armstrong, 1934; Reitman and Frankel, 1957; Fawcett and Scott, 1960; Coulombe and Favreau, 1965).
For histopathological studies of liver, kidneys, heart and lungs the tissue samples were collected separately, sliced into pieces, fixed in formalin (10%) for two days, processed, stained with Harris Hematoxylin and eosin reagent, mounted on glass slides with diphenyl xylene and observed under microscope at Bangladesh Sericulture Research Institute, Rajshahi, Bangladesh.

Results and Discussion

The structure of the compound R-1 whose toxicological studies were performed on rats in order to assess the safety of the compound is shown below:

Gross general observation: The rats of group A (control) and B (experimental) showed no sign of tremor, convulsion and reflex abnormalities. No muscular numbness of the hind and fore legs, salivation or diarrhea was observed. However, the body weights of all the rats were increased after drug administration that was found to be statistically nonsignificant (Table 1).

Table 1: Effect of the antibiotic R-1 on body weight of rats after intraperitoneal administration (n=4)

Groups	Dose (i.p.) μgm/rat/day	Body weight (g) before drug treatment $M_1 \pm SD_1$	Body weight (g) after drug treatment $M_2 \pm SD_2$	% Change	Calculated 't' value	't' value at 5% level of significance	Remark
A	300 μl	114.00±1.58	115.00±1.87	+0.877	0.580	2.447	NS
B	300 μg	111.75±1.48	112.75±1.48	+0.895	0.955	2.447	NS

M_1 and M_2 : sample mean value; SD_1 and SD_2 = standard deviations; N= number of rats; +, increase, NS= non significant

Table 2: Hematological profiles of group A rats

Hematological parameters	Normal rats 1st day	Rats treated with vehicle 7th day	Rats treated with vehicle 14th day
Total R. B. C. count (million/mm^3)	4.62±0.10	4.926±0.08	4.97±0.10
Total W. B. C. count (thousand/mm^3)	6.74±0.149	6.82±0.178	6.85±0.229
Differential count of W. B. C. (No./mm^3)			
Neutrophil	48±1.87	48±1.40	46±0.70
Lymphocyte	47.75±2.04	47.25±1.08	50.75±0.82
Monocyte	4.00±0.76	4.25±0.433	4.00±0.70
Eosinophil	0.25±0.43	0.50±0.50	0.25±0.43
Platelet count (No./mm^3)	3,51,250±24, 076	3,55,000±12, 747	3,60,000±16, 955
Hemoglobin (%)	71.25±0.829	72±0.707	72.25±0.829
E. S. R. (1st hour)	11.25±1.299	11.75±1.299	11.75±0.829

Table 3: Hematological profiles of group B rats

Hematological parameters	Normal rats 1st day	Rats treated with the compound 7th day	Rats treated with the compound 14th day
Total R. B. C. count (million/mm^3)	4.95±0.112	5.05±0.05	5.0±0.07
Total W. B. C. count (thousand/mm^3)	6.975±0.108	7.075±0.147	7.375±0.129
Differential count of W. B. C. (No./mm^3)			
Neutrophil	63.25±2.384	63.5±2.291	62.00±3.082
Lymphocyte	33.75±1.785	33.00±2.121	34.25±1.497
Monocyte	2.5±0.5	2.75±0.829	3.25±1.299
Eosinophil	1.0±0.00	0.75±0.433	0.75±0.433
Platelet count (No./mm^3)	3,00,000±7,071	3,20,000±15,811	3,07,500±14,790
Hemoglobin (%)	72.5±1.802	72.75±2.586	73.5±1.658
E. S. R. (1st hour)	13.5±1.118	13.0±1.224	11.5±1.118

Hematological profiles: No mentionable changes were found in the values of total count of RBC and WBC, differential count of WBC, platelet count, E.S.R. and hemoglobin percentage of the drug treated rats in comparison with control rats (Tables 2, 3).

Biochemical parameters of blood: Biochemical parameters of blood, e.g. SGOT, SGPT, SALP (serum alkaline phosphatase), urea and creatinine of both experimental and control rats were determined

Table 4: Effect of the antibiotic R-1 on biochemical parameters of rat's blood after i.p. administration of 300 µg/rat/day for 14 consecutive days (n=4)

Biochemical parameters	Rats of group A $M_1 \pm SD_1$	Rats of group B $M_2 \pm SD_2$	% Change	Calculated 't' values	't' values at 5% level of significance	Remark
SGPT (IU L^{-1})	8.75±0.82	8.75±0.43	0	0	2.447	NS
SGOT (IU L^{-1})	10.00±0.70	10.25±0.829	+2.50	+0.46	2.447	NS
Bilirubin (µg dl^{-1})	0.317±0.48	0.325±0.018	+2.523	+0.312	2.447	NS
SALP (IU L^{-1})	0.48±0.027	0.497±0.014	+3.541	+1.18	2.447	NS
Creatinine (mg %)	0.59±0.01	0.55±0.043	-6.779	-1.18	2.447	NS
Blood urea (m.mol L^{-1})	17.75±0.8	17.75±1.08	0	0	2.447	NS

Table 5: Effect of the antibiotic R-1 on histopathology of rat's kidney, heart, lungs and liver tissue

		Histopathological changes observed			
Groups	Dose (i.p.) µg/rat/day	Heart	Kidney	Liver	Lungs
A	300 µl vehicle	NAD	NAD	NAD	NAD
B	300µg of antibiotic	NAD	NAD	NAD	NAD

NAD indicates no abnormality detected.

after administration of the compound at a dose level of 300 μg/rat/day for 14 consecutive days to check any change of these parameters due to drug administration with respect to control rats. It was found that most of the parameters were slightly increased with respect to control animals but remained within the normal range (Table 4). From data, it was found that the changes are also statistically nonsignificant. These results indicated that the compound has no adverse effects on liver and kidney functioning.

Histopathological studies: Histopathological studies of the liver, kidneys, heart and lungs of both the control and experimental rats were performed after intraperitoneal administration of the compounds for 14 consecutive days at a dose of 300 µg/rat /day. No detectable differences in the histopathology of these organs of control and drug treated rats were observed under oil immersion objective. This indicated that the compounds have no effect on cellular structures, i.e. they do not cause degeneration of the cells of these organs (Table 5).

Discussion

In recent years owing to indiscriminate use of antibiotic the pathogenic organisms are gaining resistance to existing antibiotics and also most of the synthetic antibiotics possesses unavoidable toxic and adverse effect (Goldstein, 1974) hence the search for new, safe and more effective antimicrobial agent is a pressing need. From this point of view the present study was conducted to evaluate the safety margin of the isolated antibiotic prior to clinical trial. This study is the continuation of our previous study in which we reported the antimicrobial and toxicological study of a number of bioactive compounds isolated from plants and microbes (Rashid *et al.*, 2002; Sultan

et al., 2002; Choudury *et al.*, 2001).

The result of the present experiment indicates that although the isolated active metabolite (antibiotic) possesses slight change of haematological and biochemical parameters (at a dose of 300 µg rat^{-1} day^{-1} for 14 consecutive days) but these changes were statistically nonsignificant. No abnormalities were found in the histopathology of the liver, kidneys, heart, lungs and spleen in the experimental group. Thus the antibiotic may be considered as a safe and effective antimicrobial agent. The findings of this investigation and previous investigation (Sultan *et al.*, 2002; Choudury *et al.*, 2001) would give valuable support to make clinical trial as well as toxicity studies of the isolated antibacterial and cytotoxic metabolites to get a more potent antimicrobial agent.

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