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Sub-Acute Toxicity Study of 2-Hydroxybenzoate-deoxoserine and 2-Hydroxbenzoate-deoxo-dehydroxyserine Isolated from *Streptomyces* Species

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Abstract: The sub-acute toxicity studies of the compounds 2-hydroxybenzoate-deoxoserine and 2-hydroxybenzoate-deoxo-dehydroxyserine were carried out on Long Evan's rats using daily administration (30 μg/rat/day) of compounds for 14 consecutive days. The body weight of all rats (control and experimental) were increased after drug administration that was found to be statistically insignificant. 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 to control rats. Biochemical parameters of blood e.g. SGOT, SGPT, SALP, urea and creatinine of both experimental and control rats were found to increase slightly with respect to the control rats but remained within the normal range.

Key words: 2-hydroxybenzoate-dexoserine, 2-hydroxybenzoate-deoxo-dehydroxyserine, hematological profiles, biochemical parameters

INTRODUCTION

The challenge of developing medicine for twenty first century needs more systemic research on the branch of medicine for the welfare of human being. Toxicology is simply pharmacology at higher doses or pharmacology is simply toxicology at lower doses, which deals with the adverse effect of bioactive substances on living organisms along with their diagnosis and clinical use. Every drug has toxic effect. In order to develop and establish the safety levels of a new drug, toxicity studies are very essential. No drug is used clinically without its clinical trial as well as toxicity studies.

The production of antibiotic from genus *Streptomyces* species is well reputed (Atoni *et al*., 1997; Hamada *et al*., 1999). Following this concept, This study isolated the antagonistic strain of organism and later it was identified as *Streptomyces* species (Holt *et al*., 1994) from which two active metabolites 2-hydroxybenzoate-deoxoserine and 2-hydroxybenzoate-deoxo-dehydroxyserine having R_f value 0.311 and 0.482, respectively in the solvent system EtAc: pet ether (6.1) were isolated (Mondol, 2005).

All drugs are toxic at higher doses and even many drugs have unavoidable toxic effects at therapeutic doses. Therefore, it is important to assess the safety and efficacy of a new drug before clinically use. Therefore, keeping this objective in mind, sub-acute toxicity study of the compounds 2-hydroxybenzoate-deoxoserine and 2-hydroxybenzoate-deoxo-dehydroxyserine were carried out in Long Evan's rats to assess its effects on morphological, gross behaviour, body weight changes as well as to find out histopathological, biochemical and hematological changes (Biswas *et al*., 1998). This experiment was done at Pharmacy Lab. of Rajshahi University, in 2002.

MATERIALS AND METHODS

Collection of rats: For toxicological study, Long Evan's rats of same sex (male) and age (7 weeks) were collected from International Center for Diarrhoeal Disease Research (ICDDRB), Bangladesh.

Maintenance of the rats: The rats were kept in properly number iron cages individually in a clean animal house with an optimum room temperature and were given standard balance diet (Hawk *et al*., 1954). The rats were maintained in this way for 15 days before drug administration and continued up to end of the experiment.

Grouping of the rats: Weight of the individual rats were determined and grouped into 3 groups as such A, B and C. Each group constrains 3 rats. Group A (average body

weight 78 g) received vehicle only and acts as control group. Group B (average body weight 75 g) and Group C (average body weight 71 g) received the antimicrobial compounds 1 and 2, respectively.

Administration of the sample: The compound 1 and 2 were dissolved separately in distilled water with the help of Tween-20 in such a way that 3.5 mL contained 300 μg of the compound. The rats in group A, B and C were injected intraperitoneally with vehicle (300 μg) as well as compounds 1 (300 μg/rat/day) and 2 (300 μg/rat/day), respectively for 14 consecutive days.

Gross general observation: The body weights of each rat of both groups were measured before administration of the compounds and just prior to sacrifice them. During the whole experimental period their behaviors, CNA excitation, CNS depression, reflexes, muscular weakness, salivation and diarrhoea were monitored daily.

Experimental procedures: For hematological studies, blood was drawn from the tail vein of all the rats in groups A, B and C before administration of the compounds and blood smears were made on glass slides and stained with "Leishmen reagents" to perform TC, DC and platelet count (Ghai *et al*., 1990). With the use of capillary tubes, blood was drawn from each rat to estimate the hemoglobin percentage by "Van Kampen-Zijlstra" method (Ghai *et al*., 1990). The test was repeated on 7th and 14th day after administration of the compounds. The same procedure was followed for control rats.

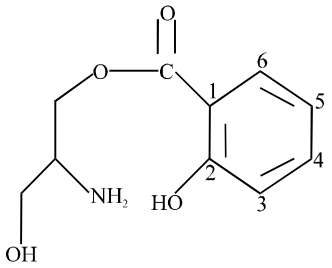
Biochemical study: Blood was collected from the throat vein of each of the rats before sacrificing them at the end of 14 days of the administration of compounds and determined SGPT, SGOT, bilirubin, creatinine, urea by using procedure and reagents as described in Englehringer Mannheim GmbH Diagnostica.

Histopathological study: Histopathological studies of heart, kidney, liver and lung were performed by staining method using hematoxylin, eosin reagent and diphenylxylene mounting fluid. The tissues were observed under microscope at Rajshahi Medical College, Rajshahi, Bangladesh.

RESULTS AND DISCUSSION

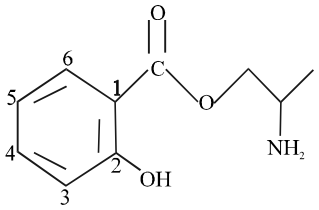
The structure of the compounds 1 and 2 whose toxicological studies were performed are given below on the basis of spectral data (Mondol, 2002).

1



2-hydroxybenzoate-deoxoserine

2



2-hydroxybenzoate-deoxo-dehydroxyserine

Gross general observation: The rats of group A, B and C showed no signs of tremor, convulsion and reflex abnormalities. No muscular numbness of hind, salivation and diarrheoa was observed. However, the body weight of all the rats were increased after compounds treatment was found to be statistically insignificant (Table 1).

Hematological profiles: No abnormalities were found in total counts of RBC and WBC, Platelet count, hemoglobin percentage and ESR of the compounds treated rats in comparison with that of control rats (Table 2).

Biochemical parameters of blood: The record of biochemical parameters in experimental groups of rats was insignificantly different in comparison with control group of rats (Table 3). These indicate that the compounds have no adverse effects on liver and kidney functioning.

Histopathological studies: Histopathological studies of the heart, kidney, liver and lung of both control and compounds treated rats showed no detectable abnormality among the 3 groups of rats, indicating that the compounds have no adverse effect on cellular structure of these organs (Table 4).

From the sub acute toxicity study, I conclude that the compounds 2-hydroxybenzoate-deoxoserine and 2-hydroxybenzoate-deoxo-dehydroxyserine have no toxic

Table 1: Effect of compounds on body weight of rats

Group of rats	Dose (i.p) rat/day	Body weight (g) before comp. Treatment n = 3, $M_1 \pm SD_1$	Body weight (g) after comp. Treatment n = 3, $M_1 \pm SD_1$	Change %	Calculated t-value	t-value at 5% level of significance	Remarks
A	300 µL	78±1.12	78.25±1.30	+0.32	0.231	2.447	NS
B	300 µL	75±1.87	77.75±1.48	+3.36	2.300	2.447	NS
C	300 µL	71±1.58	72.25±1.92	+1.76	1.518	2.447	NS

M_1 and M_2 sample mean value, SD_1 and SD_2 standard deviations; n numbers of rats; +, increase and NS, insignificant

Table 2: Hematological profiles of Group A, B and C 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	4.2±0.1	4.5±0.3	4.8±0.2	4.9±0.1	5±0.1	5.0±0.08	4.8±0.1	5.0±0.8	5±0.08
Total WBC count (thousand/cu.mm)	6.2±0.1	6.3±0.2	6.6±0.2	8±0.1	8±0.1	8±0.12	6.9±0.1	7.3±0.3	7±0.19
Differential count of WBC (no/cu.mm)									
Neutrophil 48.7±1	48.7±1	45±2	69±1.4	68±0.8	68±2	62±1	63±1.7	64±1.9	
Lymphocyte 47±1	46±1.5	50±0.7	28±2	29±1	29±1	32±1.8	32±2.3	32±1.8	
Mompocyte 4±0.7	4.5±0.5	4±0.7	0.75±0.8	1±0.7	1.7±0.8	2.7±0.82	2.7±0.8	3±0.7	
Eosinophi	0.5±0.5	0.5±0.5	0.2±0.4	1.0±0.0	1.2±0.4	1±0.7	1.0±0.0	0.75±0.4	0.75±0.4
Platelet count (milli. cu. mm)	3.4±0.1	3.1±0.1	3.6±0.1	3.2±0.9	3.3±0.04	3.3±0.02	3±0.07	3.2±0.1	3±0.1
Hemoglobin (%)	70.5±1.8	71.2±0.8	72±0.8	58±3	58±2.8	58±4	72±1.8	72±2.5	73±1.6
ESR (1st h)	10.5±1	11.5±1	12±0.7	14±1	14±1.9	15±3	13±1.1	13±1.2	11±1.1

Table 3: Biochemical parameters of Group A, B and C rats

Biochemical parameter	Group B n = 3 $M_1 \pm SD_1$	Group B n = 3 $M_2 \pm SD_2$	Charge (%)	Calculated t-value	t-values of 5% level of significance	Group C n = 3, $M_3 \pm SD_3$	Charge (%)	Calculated t-value	t-values 'of 5% level of significance
SGPT (IUL^{-1})	8.5±1.1	9±1.2	+5.8	+6.6	2.447	9.5±1.1	+11	+1.26	2.447
SGOT (IUL^{-1})	9.5±1.1	10±1.2	+3.3	+0.31	2.447	10±1.4	+7.8	+0.81	2.447
Bilirubin (µg d L^{-1})	0.30±0.4	0.31±.05	+5.8	+6.6	2.447	0.3±.05	+3.3	+0.31	2.447
Creatinine (mg%)	8.7±0.21	0.5±0.35	-32	-1.3	2.447	0.7±0.23	-16	-0.89	2.447
Blood urea (mg d L^{-1})	21.7±2.3	17±1.4	-20	-3.2	2.447	20±1.8	-5.7	-0.84	2.447

M_1, M_2 and M_3 = sample mean value; SD_1, SD_2 and SD_3 standard deviations; n, number of rats; +, increase, and - decrease., Each parameter of the above Table at 5% level of significance was non significant

Table 4: Histopathology of Group A, B and C rats

Groups	Does (i.p.) (µL/rate/day)	Histopathological changes observed Heart	Kidney	Liver	Lungs
A	300	NAD	NAD	NAD	NAD
B	300	NAD	NAD	NAD	NAD
C	300	NAD	NAD	NAD	NAD

NAD = No abnormality detected

effect in rats at the dose and duration used in this study. The results of this study may provide valuable information to researchers for further clinical trial and chronic toxicity study.

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