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Subacute Toxicity Studies of 4-hydroxy Nitrobenzene Isolated from *Streptomyces* Species

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The subacute toxicity study of an antimicrobial compound, 4-hydroxy nitrobenzene isolated from a *Streptomyces* species was carried out on long Evan's rats. The compound, 4-hydroxy nitrobenzene (300μg) was administered daily for 14 days and its effect on body weight, hematological and biochemical parameters of blood was investigated. There was no significant difference between weight gain in rats, receiving compound and control (121 $\pm$ 5.10 vs 115 $\pm$ 7.2g, respectively). There were no significant changes detected in hematology (red blood cells, 5.63 $\pm$ 0.23 vs 4.97 $\pm$ 0.17; white blood cells, 12575 $\pm$ 170.78 vs 10950 $\pm$ 238; platelet, 312500 $\pm$ 9574 vs 31000 $\pm$ 21602 (cells ml^{-1}) $\times 10^6$; hemoglobin, 13.25 $\pm$ 0.3 vs 13.27 $\pm$ 0.36%, for experimental and control rats, respectively) or blood biochemistry (serum glutamic oxaloacetic transaminase, 11.5 $\pm$ 1.29 vs 11.75 $\pm$ 1.25IU L^{-1}, serum glutamate pyruvate transaminase, 8.25 $\pm$ 1.26 vs 7.25 $\pm$ 1.5IU L^{-1}, serum alkaline phosphatase 41.25 $\pm$ 1.89 vs 44.25 $\pm$ 3.3IU L^{-1}. Uric acid, 7.05 $\pm$ 0.129 vs 7.3 $\pm$ 0.22; urea, 42.25 $\pm$ 1.708 vs 41.5 $\pm$ 2.64; creatinine, 0.85 $\pm$ 0.05 vs 1.07 $\pm$ 0.188mg dL^{-1}, for experimental and control rats, respectively). Therefore, the compound, 4-hydroxy nitrobenzene (300μg day^{-1}) over 14 days, had no toxic effects on rats.

Key words: Subacute, 4-hydroxy nitrobenzene, *Streptomyces*

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Introduction

The concept of antibiosis opened a new field of research for isolation of antibiotics from micro-organisms and so far more than 4000 such antibiotics are known. In recent years, owing to indiscriminate use of antibiotics and other unknown reasons, the pathogenic organisms are gaining resistance to existence antimicrobial and chemotherapeutic agents (Roche, 1950). Literature survey revealed that a large number of antibiotics have been isolated from the *Streptomyces* species and these species has proved to be the potential sources of antibiotics. Therefore, a *Streptomyces* species was collected and identified. An active metabolite, 4-hydroxy nitrobenzene was isolated and its antimicrobial activity was conducted (Sathi *et al.*, 2001a).

Pharmacology is a toxicology at higher doses, so, every drug shows toxicities at higher doses. Even at therapeutic doses many drugs shows unavoidable toxic effects. Moreover, toxicological data helps to make decision whether a new drug should be adapted for clinical use, for these assessments of a drug, it is necessary to investigate the toxicity to animals like, rats, guinapigs, dogs, monkeys, etc and in connection to these objectives, this work was conducted to report the toxicological studies of the compound 4-hydroxy nitrobenzene in rats.

Materials and Methods

Collection and identification of the antagonistic organism: A *Streptomyces* species (Holt *et al.*, 1994) was isolated from Rajshahi (near Fine and Arts Department of the Rajshahi University), Bangladesh, at the depth of 0.75 meter using "crowded plate technique" (Hammond and Lambert 1978).

Isolation, purification and characterization of the compound: The compound 4-hydroxy nitrobenzene was isolated from the culture filtrate of *Streptomyces* species in Yeast Extract Glucose broth medium at pH 7 after 15 days incubation at the temperature of 37.5±0.5 °C having 3% salt (NaCl) concentration. The crude compound was resolved by thin layer chromatography (TLC) and was isolated from the chloroform extract ($CHCL_3$) by preparative thin layer chromatography (PTLC) technique using solvent system $CHCl_3$: CH_3OH (10:1) .The compound was characterized on the basis of their UV, IR, NMR, Mass, HMBC and HMQC data. (Sathi *et al.*, 2001b). Purity of the compound was checked by TLC (Touchston and Dobbins, 1978) using different solvent system.

Collection, maintenance and grouping of the experimental rats: Long Evan's male rats were collected from International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B). The rats were kept in properly numbered iron cages individually in a clean animal house with an optimal room temperature (25-30^{0}C) and were given ideal food (Hawk *et al.*, 1954). The rats were maintained in this way for 15 days before drug administration and continued up to the end of the work. On the basis of weight, the rats were grouped. The rats of group B (average weight 115.5g) were used for experiment while those of group A (average weight 106.5g) were used as control.

Sample administration: The compound was dissolved in distilled water with the help of tween-20, so that 0.3ml contained 300μg of the compound. The rats in group A and B were injected intraperitoneally with vehicle (300μl) and compound, 300μg rat^{-1} day^{-1}, respectively.

Experimental Procedure: For hematological studies, blood was drawn from the tail veins of all the rats and blood smears were made on glass slides and stained with "Leishmen reagent" to perform TC (Total count), DC (differential count) and platelet count (Ghai, 1990). With the use of capillary tubes blood was drawn from each of the rat to estimate the hemoglobin percentage by "Van Kampen-Zijlstra's" method (Ghai, 1990).

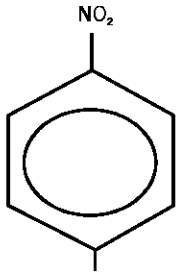
For the study of biochemical parameters such as SGOT (Serum glutamate oxaloacetate transaminase), SGPT (Serum glutamate pyruvate transaminase), serum alkaline phosphatase and serum creatinine, uric acid and urea were determined by using the procedures and reagents described in "Enlehringer Mannheim GmbH Diagnostica" (king and Armstrong, 1934; Reitman and Frankel, 1957; Fawcett and Scott, 1960; Coulombe and Favreau, 1963).

For histopathological study, liver, kidney, heart, lungs and spleen of all of the rats tissues were sliced into pieces and immersed in 10% formalin for three days, processed, stained with "Harris Hematoxylin and eosin reagent", mounted on glass slides with diphenyl xyline mounting fluid and observed under microscope at the Bangladesh Sericulture Research Institute, Rajshahi, Bangladesh.

Statistical Analysis: Results are presented as the mean ± S.D, Students t-test was used for comparison between the experimental and control groups. $p<0.05$ was considered to be statistically significant (Gujarati, 1988).

Results and Discussion

The structure of the compound, used for toxicological study is shown below:



4-hydroxy nitrobenzene

Table 1: Body weight after daily intraperitoneal administration of 4-hydroxy nitrobenzene (300μg) for 14 days in rats

Group	Dose(i.p.) 300μg rat^{-1} day^{-1}	Body weight (gm) before drug treatment n = 4, M_1 ±SD_1	Body weight(gm) after drug treatment n = 4, M_2 ±SD_2	% change	Calculated "t" value	value at 5% level of significance
A	300 μl vehicle	106.5±2.65	115±7.12	+7.98	+2.24	2.447 NS
B	300 μg compound	115.5±6.56	121±5.10	+4.76	+1.32	2.447 NS

M_1 and M_2 indicate sample mean values, SD_1 and SD_2 = Standard deviations, n = Number of rats, + = Increase, NS = Not Significant

Table 2: Hematological profiles after daily intraperitoneal administration of 4-hydroxy nitrobenzene (300μg) for 14 days in rats

Parameters	Control			Experimental		
(cells mL^{-1}) × 10^6	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
RBC	4.85±0.21	5.63±0.236	4.97±0.17	4.97±0.17	4.73±0.22	5.63±0.23
WBC	11400±141	10800±216	10950±238	12457±250	12700±244.9	12575±170.78
Neutrophils	39.5±1.29	36.5±1.29	37.75±0.957	42.5±2.08	41.5±1.29	41.5±2.38
Lymphocytes	52.25±0.95	54.75±0.957	53.75±1.26	47.5±1.94	49.25±1.7	50±1.41
Monocytes	4.5±1.0	4.75±1.5	4.5±1.29	5.75±0.5	5±0.816	4.75±0.96
Eosinophils	3.75±0.5	4.0±0.816	4.0±0.816	4.25±0.5	4.25±0.96	3.75±0.5
Platelet	322500 ±10408.33	332500 ±22173.56	310000 ±21602	340000 ±14142	297500±50000	312500 ±9574
Hemoglobin (%)	13.65±0.3	13.77±0.126	13.27±0.368	12.8±0.47	12.98±0.33	13.25±0.3

Values are mean ± SD, n =4

Table 3: Biochemical parameters after daily intraperitoneal administration of 4-hydroxy nitrobenzene (300μg) for 14 days in rats

Parameters	Control	Experimental	% Change
SGOT (IUL^{-1})	11.75±1.25	11.5±1.29	-2.13
SGPT (IUL^{-1})	7.25±1.5	8.25±1.26	+13.79
SALP (IUL^{-1})	44.25±3.30	41.25±1.89	-6.78
Serum uric acid (mg dl^{-1})	7.3±0.22	7.05±0.129	-3.42
Urea (mg dl^{-1})	41.5±2.64	42.25±1.708	+1.8
Serum creatinine (mg dl^{-1})	1.07±0.188	0.85±0.05	-20.63

Values are mean ± SD
SGPT : Serum glutamate pyruvate transaminase
SALP : Serum alkaline phosphatase
SGOT: Serum glutamic oxaloacetic transaminase

Body weight: Table 1 showed the individual and average body weights of all the rats before and after drug administration. The body weight of all the rats increased after the drug administration, which were found to be statistically insignificant.

Hematological profiles: Hematological profiles were done to check the hematological abnormalities after intraperitoneal administration of the test sample. The changes in values of total count of RBC and WBC, differential count of WBC, platelet count and hemoglobin percentage before and after sample administration were very slight (Table 2). However, the changes with in the normal range (Islam *et al.*, 1997)

Biochemical Parameters of blood: Biochemical parameters of blood e.g. SGOT, SGPT, SALP (Serum Alkaline Phosphatase), urea, uric acid and serum creatinine of both experimental and control rats, were determined to check any change of the parameters due to administration of the compound with respect to the control rats (Table 3). It was found that most of the parameters were slightly increased with respect to the control but remained with in the normal range. The results also revealed that the changes were statistically insignificant. These results indicated that the compound has no adverse effect on liver and kidney functioning (Anisuzzaman *et al.*, 2001)

Histopathological Studies: After 14 day of drug treatment, the animals of both control and experimental groups were sacrificed and the organs such as liver, kidney, lung, spleen and heart were isolated and histopathological examinations were done. No detectable abnormality was observed between the control and the drug treated rats, indicating that the compound, 4-hydroxy nitrobenzene has no adverse effect on cellular structure.

From the experiment, conclude that the compound, 4-hydroxy nitrobenzene isolated from *Streptomyces* species has no toxic effects in rats at 300μg rat^{-1} day^{-1} for 14 day treatment.

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