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Sub-acute Toxicity Study of 3,6-Dimethyl-4-Ethyl-O-Acetyl Benzene Isolated from Soil *Streptomyces* Species

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Abstract: The sub-acute toxicity study of 3,6-dimethyl-4-ethyl-0-acetyl benzene (BM-5), was carried out on Long Evan's rats using daily administration (300 µg/rat/day) of compound for 14 consecutive days. Non-significant differences between weight of compound receiving rats and control rats (48±0.816 vs 46±0.816) were found. The change in hematological parameters was found to be nonsignificant (total count of RBC, 5.1±0.081 vs 4.63±0.094; white blood cell, 7.33±0.124 vs 5.73±0.124; platelets, 361666±10274 vs 261666±8498, (cell/ml) $\times 10^6$; percentage of hemoglobin 65.67±1.60 vs 56±0.816; ESR, 25±0.816 vs 19.67±0.471 for experimental and control rats, respectively) and biochemical parameters (serum glutamate pyruvate transaminase, 8.5±0.408 vs 8.33 ±0.471 IU L^{-1}; serum glutamate oxaloacetate transaminase, 9.83±0.235 vs 9.33±0.234 IU L^{-1}; bilirubin, 0.36±0.104 vs 0.34±0.016 µg dL^{-1}; creatinine, 0.67 ±0.089 vs 0.61±0.009 mg dL^{-1}; urea, 18.83±0.235 vs 18.5±0.408 mg dL^{-1}; for experimental and control rats). Therefore, the changes in body weight, hematological and biochemical parameters were statistically non-significant. No detectable abnormalities were found in histopathology of heart, kidney, liver and lung in experimental group of rats as compared with that of the control group of rats.

Key words: 3,6-dimethyl- 4-ethyl- 0- acetyl benzene, *Streptomyces* species, sub-acute toxicity

Introduction

The challenge of developing modern medicine for twenty first century needs more systemic research on the branch of medicine for welfare of the humanity, because microbial drug resistance is one of the serious problems, which the human race is facing today. Therefore, the worldwide increasing demand for medicine from natural sources has motivated search for new drugs with high potential activity.

The production of antibiotic from genus *Streptomyces* species is well reputed (Atoni *et al.*, 1997; Hamada *et al.*, 1999; Anisuzzaman *et al.*, 2001). Based on this concept, we isolated an antagonistic strain of organism and later it was identified as *Streptomyces* species (Holt *et al.*, 1994) from which active metabolite 3,6-dimethyl-4-ethyl-0-acetyl benzene was isolated having R_f value 0.61 in solvent ($CHCl_3$: MeOH, 12:1).

In order to assess the safety and efficacy level of a drug, toxicity studies are very essential.

Toxicological data help to make decision whether a new drug should be clinically used or not. Therefore keeping this objective in view, we herein, report the toxicological studies of the compound 3,6-dimethyl-4-ethyl-0-acetyl benzene (BM-5) in rats.

Methods and Materials

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

Maintenance of the rats: The rats were kept in properly numbered iron cages individually in a clean animal house with an optimum room temperature and were given standard balanced diet (Hawk *et al.*, 1954). The rats were maintained by this way for 15 days prior to administration of the compound and continued until completion of the experiment.

Grouping of the rats: Rats were weighed individually and divided into two groups; group-A (average body weight, 45 g) and group-B (average body weight 46.5 g), each comprising of three rats. Group A is used as a control.

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 rats in group A and B were injected intra-peritoneally with vehicle (300 µl) and compound 300 µg/rat/day respectively for 14 consecutive days.

Gross general observation: The body weight of each rat of both groups was taken before administration of the compound and just prior to sacrifice them. During the whole experimental period their behaviour, CNS excitation, CNS depression, reflexes, muscular weakness, salivation and diarrhoea were monitored daily.

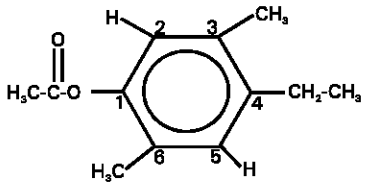
Experimental procedure: For hematological studies, blood was drawn from the tail vein of rats of both the groups before the administration of the compound and blood smears were made on glass slides and stained with "Leishmen reagents" to perform TC, DC and Platelet count (Ghai *et al.*, 1990). With the use of capillary tubes, blood was drawn from each rat to estimate the hemoglobin percentage by "Van Kampen-Zijlstra's" method (Ghai *et al.*, 1990). The test was repeated on 7th and 14th day after administration of the compound.

For biochemical study, blood was collected from the throat vein of each of the rats after sacrificing them at the end of 14 days of the administration of compound and determined SGPT, SGOT, bilirubin, creatinine, urea by using procedure and reagents as described in Enlehringer Mannheim GmbH Diagnostica (King and Armstrong, 1934; Reitman and Frankel, 1957; Coulomble and Favreau, 1963).

Histopathological studies of heart, kidney, liver and lung were performed by staining method using hematoxylin, eosin reagent and diphenyl xylene mounting fluid. The tissues were observed under microscope at the Department of Genetics and Breeding, University of Rajshahi, Bangladesh.

Results and Discussion

The structure of the compound whose toxicological studies were performed is shown below on the basis of spectral data (Bytul, 2002).



3,6- dimethyl- 4-ethyl- O-acetyl benzene

Gross general observation: The rats of group A and B showed no signs of tremor, convulsion and reflex abnormalities. No muscular numbness of hind, salivation and diarrhoea was observed. However, the observed changes in body weight before and after drug treatment were found statistically non significant (Table 1).

Table 1: Effect of compound on body weight of rats

Group of rats	Dose (i.p.) μg/rat/day	Body weight (g) before drug treatment n=3, $M_1 \pm SD_1$	Body weight (g) after drug treatment n=3, $M_2 \pm SD_2$	% Change	Calculated 't' value	't' value at 5% level of significance	Remarks
A	300 μl	45.0±0.816	46±0.816	+2.222	1.500	2.776	NS
B	300 μg	46.5±0.707	48±0.816	+3.225	1.656	2.776	NS

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

Hematological profiles: No abnormalities were found in total counts of RBC and WBC, platelet count, hemoglobin percentage and ESR of the drug 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 is non-significantly different in comparison with control group of rats (Table 3). These indicate that the compound has no adverse effects on liver and kidney functioning (Anisuzzaman *et al.*, 2001).

Histopathological studies: Histopathological studies of the heart, kidney, liver and lung of both control and drug treated rats (Table 4) showed no detectable abnormality between the two groups

Table 2: Hematological profiles after i.p. administration of compound (300 µg/rat/day) for 14 days in control and compound receiving rats

	Control			Experimental		
Hematological parameters	1st day	7th day	14th day	1st day	7th day	14th day
Total R. B. C. count (million/cu. mm)	4.43±0.124	4.6±0.0816	4.63±0.094	5±0.081	5.03±0.047	5.1±0.081
Total W. B. C. count (thousand/cu. mm)	5.7±0.163	5.63±0.094	5.73±0.124	7.03±0.047	7±0.081	7.33±0.124
Differential count of W. B. C. (%)						
Neutrophil	60.33±1.70	60±1.632	59±0.816	60.67±1.70	59.67±2.867	61.67±2.054
Lymphocyte	35.33±1.247	35±1.632	36.67±0.942	37.67±1.247	38.33±2.054	37±2.160
Monocyte	1±0.816	1±0	1.33±0.471	0.33±0.471	0.67±0.471	0.33±0.471
Eosinophil	3±0.816	3±0	2.67±0.471	1.33±0.471	1.33±0.471	1±0
Platelet count (No./cu. mm)	256666±12472	251666±10274	261666±8498	363333±12472	358333±13123	361666±10274
Hemoglobin (%)	54.0±0.471	55.67±0.471	56 ± 0.816	68 ± 0.816	65.33±1.247	65.67±1.60
E. S. R. (1st hour)	23.33±0.471	24.33±0.942	25 ± 0.816	18.6 ± 0.471	18.7±0.471	19.67±0.471

Table 3: Effect of compound on the biochemical parameters of rat's blood after i.p. administration of 300 µg/rat/day for 14 consecutive days

Biochemical parameters	Control rats (group A) n= 3, M_1± SD_1	Experimental rats (group B) n= 3, M_2± SD_2	% Change	Calculated 't' values	't' values at 5% level of significance	Remarks
SGPT (IU L^{-1})	8.33±0.471	8.5±0.408	+2.041	0.555	2.776	NS
SGOT (IUL^{-1})	9.33±0.234	9.83±0.235	+5.359	2.611	2.776	NS
Bilirubin (µg dL^{-1})	0.34±0.016	0.36±0.014	+5.882	1.629	2.776	NS
Creatinine (mg %)	0.61±0.009	0.67±0.089	+9.836	1.161	2.776	NS
Blood urea (mg dL^{-1})	18.5±0.408	18.83±0.235	+1.783	1.213	2.776	NS

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

Table 4: Effect of the compound on histopathology of rat's heart, kidney, liver and lungs tissue i.p. administration of 300 µg/rat/day for 14 consecutive days

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

NAD = No abnormality detected.

of rats, indicating that the compound has no adverse effect on cellular structures of these organs (Anisuzzaman *et al.*, 2001).

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 drug. Form the subacute study, we conclude that the compound 3,6-dimethyl-4-ethyl-0-acetyl benzene has no toxic

effect in rats at the dose and duration used in this study and the compound is suitable for further clinical trial.

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