

## Sub-acute Toxicity Study of 2-Hydroxy-3(Hydroxymethyl)- 4H-Pyran-4-One Isolated from Soil *Streptomyces* Species

<sup>1</sup>Bytul M. Rahman, <sup>1</sup>M. Abdul Alim Al-Bari, <sup>1</sup>M. Mukhlesur Rahman,  
<sup>1</sup>M. Robiul Islam and <sup>2</sup>M. Shah Alam Bhuiyan

<sup>1</sup>Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

<sup>2</sup>Department of Biology, Indiana University-Purdue University  
Indianapolis (IUPUI), 723 West Michigan St. Indianaapolis, IN 46202, USA.

**Abstract:** The sub-acute toxicity study of 2-hydroxy-3(hydroxymethyl)- 4H-pyran-4-one (BM-4) isolated from soil *Streptomyces* species was studied on Long Evan's rats using daily administration in a dose of 300 µg/rat/day for consecutive 14 days. The studies included the gross general observation and non-significant difference between weight of compound receiving rats and control rats (48.57 ±0.935 vs 46 ±0.816) were found. The studies were carried out in haematological profiles [total count of Red Blood Cells (RBC), 5.1 ±0.081 vs 4.63 ±0.094; White Blood Cells (WBC), 6.07 ±0.094 vs 5.73 ±0.124; Differential count of WBC in %; platelets, 301666 ±14337 vs 261666 ±8498 (cell/ml x 10<sup>6</sup>); percentage of hemoglobin (Hb%), 59.67 ±1.247 vs 56 ±0.816; ESR, 14.67 ±0.471 vs 25 ±0.816; for experimental and control rats respectively] and biochemical profiles of blood [Serum Glutamate Pyruvate Transaminase (SGPT), 8.43 ±0.329 vs 8.33 ±0.471; Serum Glutamate Oxaloacetate Transaminase (SGOT), 9.5 ±0.244 vs 9.33 ±0.234; bilirubin, 0.36 ± 0.008 vs 0.34 ±0.016; urea, 18.83 ±0.235 vs 18.5 ±0.408; creatinine, 0.63 ±0.009 vs 0.61 ±0.009; for experimental and control rats respectively] and also in the histopathology of the liver, kidney, heart and lung of both control and experimental groups of rats. The changes in body weight, hematological and biochemical parameters were statistically not significant. No detectable abnormalities were found in the histopathology of the liver, kidney, heart and lung in the experimental groups of rats following same dose when compared with control group of rats. This preliminary study suggests that the isolated compound may be used safely for clinical trial.

**Keywords:** 2-hydroxy-3(hydroxymethyl)- 4H-pyran-4-one, Sub-acute toxicity, *Streptomyces* species

### INTRODUCTION

For the well being of mankind one of the major contributions of science and research has been the isolation of variety of antibiotics from microorganism and their use as chemotherapeutic agents for the treatment of infectious diseases. The growth in antibiotic usage globally has been paralleled by the ability of bacteria to resist being killed by these agents and has resulted in a steady decline in the number of effective antibiotic each year. Although medical scientists are discovering newer and more potent antimicrobial drugs, the pathogenic microbes with their determination to survive are gaining resistance by curious mechanisms. Indeed, microbial drug resistance is one of the most serious problems, which the human race is facing today. Therefore, the challenge of developing modern medicine for twenty first century needs more systemic research on the branch of medicine for welfare of the humanity. In this intention the present study was designed to isolate and

characterize antibiotic as well as to observe its biological activities<sup>[1]</sup>. Soil organisms could provide a rich source of antibiotics<sup>[2]</sup>. The production of antibiotics from genus *Streptomyces* species is well reputed<sup>[3,4,5,6]</sup>. As part of such efforts on the microbial metabolites from soil sample of Bangladesh<sup>[7,8,9]</sup>, we isolated an antagonistic *Streptomyces* species<sup>[10]</sup>. From isolated soil *Streptomyces* species, we have isolated an active metabolite 2-hydroxy-3(hydroxymethyl)- 4H-pyran-4-one and the structure of the metabolite was confirmed by chemical and spectroscopic techniques including IR, Positive and negative mode of mass spectra, high resolution <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, HMQC and HMBC<sup>[11]</sup>. The antimicrobial activity profile and MIC level of 2-hydroxy-3(hydroxymethyl)- 4H-pyran-4-one was interesting against some pathogenic bacteria<sup>[11]</sup>.

In order to assess the safety and efficacy level of a new drug, toxicological studies are very essential experiments in animals like rat, guinea pigs, dog, monkey etc under various conditions of drug. 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. This led to the present investigation on the sub-acute toxicity study of the compound 2-hydroxy-3(hydroxymethyl)-4H-pyran-4-one (BM-4) at a dose of 300 µg/day/rat on 6 Long Evan's rats for 14 consecutive days.

## MATERIALS AND METHODS

The present study was carried out during January to July' 2002 in Pharmaceutical Microbiology Laboratory, Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh.

### Collection and Maintenance of Experimental Rats:

6 Long Evan's male rats of about seven weeks old were collected from the Animal Branch of International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The rats were kept in properly numbered iron cages individually in a hygienic animal house with an optimum room temperature (25-30 °C) and were given standard laboratory diet<sup>[12]</sup>. The rats were maintained in this way for 15 days prior to drug administration and continued up to the end of the experiment.

**Grouping of the Rats:** Individual weight of the rats was taken and they were grouped into two. The rats of group B (3 rats, average weight 48 g) were used for experiment while those of group A were used as control (3 rats, average weight 45 g).

**Administration of the Sample:** The compound was dissolved in distilled water with the help tween-20 as co-solvent, so that 0.3 ml contained 300 µg of the compound. The rats in group A and B were injected intraperitoneally with vehicle (300 ml isotonic solution) and compound respectively at a dose of 300 mg/day/rat for 14 consecutive days. On the 15<sup>th</sup> day blood was collected from external jugular vein under mild ether anesthesia for the estimation of hematological and biochemical parameters. Then all the rats were sacrificed and liver, kidney, heart and lung were removed for histopathological study.

**Gross General Observation:** The body weight of each rat of groups A and B were measured before drug administration, after completion of the treatment and just prior to sacrificing the rats. During the whole experiment period their behaviour, CNS excitation and depression, reflexes, muscular weakness, salivation, diarrhoea, and food intake were observed.

**Experimental Procedure:** For Investigation of haematological profiles, blood was drawn from the tail

veins of the experimental and the control rats of either groups just before drug intake and blood smears were made on glass slides followed by staining with "Leishman reagent" to performed Total Count (TC) of RBC and WBC, Differential Count (DC) of WBC and platelet count<sup>[13]</sup>. Blood was also drawn from each rat with the help of capillary tube for estimating the haemoglobin percentage by Van Kampen-Ziftra's method<sup>[13]</sup>. The test was repeated on 7<sup>th</sup> and 14<sup>th</sup> day after administration of the compound.

For biochemical study, blood samples were collected from the throat vein of each rat of group while they were sacrificed after 14 days of drug administration and determined the parameters such as SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase), serum bilirubin, creatinine and urea by using the usual procedures and reagents as described in Enlehringer Mannheim GmbH Diagnostica<sup>[14,15,16]</sup>.

Histopathological investigation of liver, kidney, lung and heart of all experimental and control rats were performed after sacrificing them at the end of 14 days of drug administration. These tissues were separately sliced in pieces, fixed in 10% formaline for 3 days; processed; stained using hematoxylin, eosin reagent and diphenyl xylene mounting fluid; mounted on glass slides and observed under power microscope at the Department of Genetics and Breeding, University of Rajshahi, Bangladesh.

## RESULT AND DISCUSSIONS

The rats of group A and B being treated with vehicle and compound respectively exhibited no signs of tremor, convulsions and reflex abnormalities. The observed changes in body weight before and after drug treatment were found statistically non significant (Table 1). Moreover, no muscular numbness of the hind and fore legs, salivation or diarrhoea was observed. The food intake per day was also found normal. So, from the results, it is decided that the drug has no effect on normal growth.

Table 2 and 3 showed haematological profiles that were studied on experimental rats in comparison with that of control rats and 14 days of treatment. Each time the value of the parameters in each rat was changed slightly and which were non significant.

The record of biochemical parameters in treatment groups of rats were non significantly different in comparison with control group of rats (Table 4). The parameters remained within the normal range. This indicates that the compound 2-hydroxy-3(hydroxymethyl)-4H-pyran-4-one have no adverse effect on liver and kidney functioning<sup>[6]</sup>.

**Table 1:** Effect of the compound on body weight of rats

Group	Dose (i.p.) $\mu\text{g}/\text{rat}/\text{day}$	Body weight (gm) before drug administration $n=3$ . $M_1 \pm SD_1$	Body weight (gm) after drug administration $n=3$ . $M_2 \pm SD_2$	% of change	$t_c$	$t_s$	Remark
A	300 mL vehicle	45, 46, 44 $45 \pm 0.816$	46, 47, 45 $46 \pm 0.816$	+2.222	1.500	2.776	NS
B	300 mg compound	47, 48, 49 $48 \pm 0.816$	47.5, 49, 49.57 $48.57 \pm 0.935$	1.562	1.046	2.776	NS

$t_c$  indicates calculated value;  $t_s$  indicates t value at 5% level of significance;  $M_1$  and  $M_2$ , sample mean value;  $SD_1$  and  $SD_2$ , standard deviation; n, number of rats; NS, not significant; +, increase.

**Table 2:** Haematological profiles of group A (rat treated with vehicle)

Haematological parameters	Normal rat			Treated with vehicle						
	1 <sup>st</sup> day $M_1 \pm SD_1$			7 <sup>th</sup> day $M_2 \pm SD_2$			14 <sup>th</sup> day $M_3 \pm SD_3$			
Total RBC count (million/cc)	4.3, 4.4, 4.6 $4.43 \pm 0.124$			4.5, 4.6, 4.7 $4.6 \pm 0.0816$			4.5, 4.7, 4.7 $4.63 \pm 0.094$			
Total WBC count (thousand/cc)	5.5, 5.7, 5.9 $5.7 \pm 0.163$			5.5, 5.7, 5.7 $5.63 \pm 0.094$			5.6, 5.7, 5.9 $5.73 \pm 0.124$			
Differential count of WBC in %	a. Neutrophil	58, 61, 62 $60.33 \pm 1.70$			60, 62, 58 $60 \pm 1.632$			60, 59, 58 $59 \pm 0.816$		
	b. Lymphocyte	37, 34, 35 $35.33 \pm 1.247$			35, 33, 37 $35 \pm 1.632$			36, 36, 38 $36.67 \pm 0.942$		
	c. Monocyte	0, 2, 1 $1 \pm 0.71$			1, 1, 1 $1 \pm 0$			1, 2, 1 $1.33 \pm 0.471$		
	d. Eosinophil	4, 3, 2 $3 \pm 0.816$			3, 3, 3 $3 \pm 0$			3, 3, 2 $2.67 \pm 0.471$		
Platelet count (no./cc)	240000, 270000, 260000 $256666 \pm 12472$			250000, 240000, 260000 $251666 \pm 10274$			250000, 260000, 270000 $261666 \pm 8498$			
Haemoglobin (%)	54, 55, 55 $54.66 \pm 0.471$			55, 56, 56 $55.67 \pm 0.471$			55, 57, 56 $56 \pm 0.816$			
ESR (mm/1 <sup>st</sup> hour)	23, 23, 24 $23.33 \pm 0.471$			25, 23, 25 $24.33 \pm 0.942$			25, 24, 26 $25 \pm 0.816$			

**Table 3:** Haematological profile of group B (rat treated with Compound, BM-4)

Haematological parameters	Normal rat			Treated with compound 1						
	1 <sup>st</sup> day $M_1 \pm SD_1$			7 <sup>th</sup> day $M_2 \pm SD_2$			14 <sup>th</sup> day $M_3 \pm SD_3$			
Total RBC count (million/cc)	4.9, 5.0, 5.2 $5.03 \pm 0.124$			5.2, 4.9, 5.3 $5.13 \pm 0.170$			5.1, 5.0, 5.2 $5.1 \pm 0.081$			
Total WBC count (thousand/cc)	6.1, 6.0, 5.7 $5.93 \pm 0.17$			5.9, 5.8, 6.0 $5.9 \pm 0.082$			6.0, 6.0, 6.2 $6.07 \pm 0.094$			
Differential count of WBC in %	a. Neutrophil	54, 60, 59 $57.67 \pm 2.624$			54, 58, 59 $57 \pm 2.160$			55, 58, 60 $57.67 \pm 2.054$		
	b. Lymphocyte	42, 37, 38 $39 \pm 2.160$			44, 38, 39 $40.33 \pm 2.624$			42, 40, 37 $39.67 \pm 2.054$		
	c. Monocyte	1, 1, 2 $1.33 \pm 0.471$			1, 1, 2 $1.33 \pm 0.471$			2, 0, 1 $1 \pm 0.816$		
	d. Eosinophil	3, 1, 1 $1.67 \pm 0.942$			1, 3, 0 $1.33 \pm 1.247$			1, 1, 2 $1.33 \pm 0.471$		
Platelet count (no/cc)	310000, 270000, 275000 $285000 \pm 17795$			280000, 300000, 278000 $286000 \pm 9933$			300000, 320000, 285000, 248000 $301666 \pm 14337$			
Haemoglobin (%)	57, 58, 60 $58.33 \pm 1.247$			58, 61, 61 $60 \pm 1.414$			60, 58, 61 $59.67 \pm 1.247$			
ESR (mm/1 <sup>st</sup> hour)	14, 16, 12 $14 \pm 1.632$			14, 17, 14 $15 \pm 1.414$			15, 14, 15 $14.67 \pm 0.471$			

**Table 4:** Effect of compound BM-4 on biochemical parameters of rat's blood after i.p. administration of 300  $\mu\text{g}/\text{rat}/\text{day}$  for 14 consecutive days.

Biochemical parameters	Control rats, Group A, $n=3$ $M_1 \pm SD_1$	Experimental rats, Group B, $n=3$ $M_2 \pm SD_2$	% of change	$t_c$	$t_s$	Remark
SGPT (IUL <sup>-1</sup> )	8, 9, 8 $8.33 \pm 0.471$	8, 8.8, 8.5 $8.43 \pm 0.329$	1.2	0.301	2.776	NS
SGOT (IUL <sup>-1</sup> )	9.5, 9, 9.5 $9.33 \pm 0.234$	9.8, 9.2, 9.5 $9.5 \pm 0.244$	1.82	0.869	2.776	NS
Serum bilirubin (mmolL <sup>-1</sup> )	0.35, 0.32, 0.36 $0.34 \pm 0.016$	0.37, 0.35, 0.36 $0.36 \pm 0.008$	5.882	1.936	2.776	NS
Creatinine (mg %)	0.6, 0.62, 0.6 $0.61 \pm 0.009$	0.62, 0.62, 0.64 $0.63 \pm 0.009$	5.278	2.721	2.776	NS
Urea (mmolL <sup>-1</sup> )	18, 19, 18.5 $18.5 \pm 0.408$	18.5, 19, 19 $18.83 \pm 0.235$	1.783	1.213	2.774	NS

'NS' indicates not significance

**Table 5:** Effect of compound BM-4 on histopathology of rat's kidney, heart, liver and lung tissue after i.p. administration of 300 µL/rat/day for 14 consecutive days.

Group	Dose (i.p.) mL/rat/day	Observation of histopathological change			
		Kidney	Liver	Heart	Lung
A	300 mL(Vehicle)	NAD	NAD	NAD	NAD
B	300 mL(Compound BM-4)	NAD	NAD	NAD	NAD

NAD = No Abnormality Detected.

No detectable abnormality was observed between the control and the drug treated rats when the tissue slides of liver, kidney, lung and heart were examined under microscope which indicated the compound have no adverse effect on cellular structure i.e., the compound does not cause degeneration of cells of these organs (Table 5).

The results of present study demonstrate that the compound, 2-hydroxy-3(hydroxymethyl)- 4H-pyran-4-one possess no adverse effect on Long Evan's rats at a dose of 300 µg/rat/day. Thus the findings of this investigation would give valuable support to make clinical trial of the isolated compound.

#### ACKNOWLEDGEMENT

The authors are pleased to acknowledge Professor Dr. Md. Abdur Rashid, Department of Pharmacy, University of Dhaka, Bangladesh for producing spectral analysis and Dr. Anwar Habib, Assistant Professor, Department of Pharmacology, Rajshahi Medical College, Rajshahi, Bangladesh for his cooperation and help in studying subacute toxicity study of the compound.

#### REFERENCES

- Bytul, M. Rahman, 2002. Studies on a soil *Streptomyces* species and its bioactive metabolites. M. Pharm. Thesis, Department of Pharmacy, University of Rajshahi, Bangladesh.
- Arnold, R. Martin, 1992. Antibiotics, Wilson and Gisvolds, Text book of organic and pharmaceutical chemistry, 9<sup>th</sup> edition, pp: 227,314,337.
- Imai, S., A. Shimazu, K. Furihata, Y. Hayakawa and H. Seto, 1990. Isolation and structure of a new phenoxazine antibiotic, expoliazone, produced by *Streptomyces exfoliates*. J. Antibiotics, 43(12): 1606-7.
- Atoni, Y., H. Nagata and M. Yoshido, 1997. Novel immunosuppressant from *Streptomyces* species, J Antibiotic Tokyo, 50: 543-545.
- Hamada, M. and S. Yamamoto, 1999. Conagenin derived from *Streptomyces roseosporus* macrophage functions, J Antibiotic Tokyo, 52: 548-551.
- Anisuzzaman, A.S.M., N.Sugimoto, S.A. Bhuiyan, G. Sadik and M.A. Gafur, 2001. Characterization and in vitro antimicrobial activity of the two novel compounds of *Streptomyces* species. The Sciences, 1: 220-223.
- Jabbar, A., A. Hasnat, S.A. Bhuiyan, M.A. Rashid and S. Reza, 1995. Isolation and *in vitro* antibacterial screening of a tricarboxylic acid anhydride from *Penicillium* sp. Pharmazie, 50. H. 10.
- Biswas, M.S.A., A.R.M.R. Amin, M.A. Islam, C.M. Hasan, K.R. Gustafson, M.R. Boyd, L.K. Pannel and M.A. Rashid, 2000. Monocillinols A and B, novel fungal metabolites from a *Monocillium* species. Tetrahedron Letter, 41: 7177-7180.
- Bhuiyan, M.S.A., M.S. Reza and A. Jabbar, 2002. Antibacterial activity of *Bacillus* species from a contaminated culture plate. Dhaka Univ. J. Biol. Sci., 11(1): 105-108.
- Holt. J.G., N.R. Krieg, P.H.A. Sneath, J.T Staley and S.T. Williams, 1994. Bergey's Manual of Determinative Bacteriology. 9<sup>th</sup> edn., pp: 307-308.
- Bytul, M.R., M.S.A. Bhuiyan, M.A.A. Al-Bari and A.S.M Anisuzzaman, 2002. Susceptibility of crude metabolites and two isolated compounds of soil *Streptomyces* species to pathogenic organisms. Journal of Medical Sciences, 1(5): 237-239.
- Hawak, P.B., L. Oser and W.H. Summerso, 1954. Practical Physiological Chemistry. 13<sup>th</sup> edn. McGraw Hill Book Company USA.
- Ghai, C.L., 1990. A test book of practical physiology, Jaypee Brothers (p) Ltd. India, pp: 119-202.
- King, P.J.E. and A.R. Armstrong, 1934. A convenient method for determination serum and bile phosphatase activity. Cand. Med. Assoc., 31: 376-381.
- Reitman, S. and S. Frankel, 1957. A calorimetric method for the determination of serum glutamate oxalate and glutamate pyruvate transaminase. Am. J. Clin. Pathol., 28: 56-63.
- Columbe, H. and I. Favreau, 1963. A new simple semi micro method for determination of urea. J. Clin. Chem., 9: 107-108.