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Sub-acute Toxicity Study of 5-Hydroxy - 2(Hydroxy-Methyl) 4H-pyran-4 One, Isolated from *Aspergillus fumigatus*

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Abstract: The sub-acute toxicity studies of 5-Hydroxy-2 (Hydroxy-methyl) 4H- pyran-4 one, a metabolite of *Aspergillus fumigatus* was carried out in rats. The compound was administered at a dose of 300 μg/rat/day for 21 days. The gross general observation such as changes of body weight, hematological profiles, biochemical parameters of blood and the histopathology of the liver, kidney, heart, lung, spleen 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 insignificant. No abnormalities were found in the histopathology of the liver, kidney, heart, lung and spleen in the experimental group of rats when compared with control group of rats.

Key words: *Aspergillus fumigatus*, 5-Hydroxy-2 (Hydroxy methyl)- 4H-pyran-4 one, toxicity

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. In recent years, owing to indiscriminate use of antibiotics and other unknown reasons the pathogenic organisms are gaining resistance to existing antimicrobial agents (Roche, 1950). Hence the search for new, safe and more effective antibiotics against these organisms is a pressing need. Still now, the richest sources of known fungal antibiotics are the species of the genera *penicillium* and *Aspergillus* of the family Aspergillaceae (Gennaro, 1990). Therefore, A*spergillus fumigatus* was collected and identified. An active metabolite, 5–Hydroxy–2 (Hydroxy methyl) 4H-pyran- 4 one was isolated and its antimicrobial screening was conducted by Anisuzzaman (2000).

In order to develop and to 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 helps to make decision whether a new drug should be 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 5- hydroxy-2 (hydroxy-methyl)- 4H – pyran – 4 one in rats.

Materials and Methods

Collection of experimental rats: Long Evan's rats of same sex (male, 10) and age (7 weeks) were collected from International Center for Diarrhoeal Diseases Research, Bangladesh (ICDDR, B).

Maintenance of the rats: The rats were housed in a clean room with an optimal room temperature and were caged individually with proper marking. The animals were maintained on standard balanced diet for 15 days prior to administration of 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 111.3 gm/rat) and group B (average body weight 116.2 gm/rat), each comprising of 5 rats. Group A received vehicle only to act as control, while group B received the compound.

Administration of the sample: The compound was dissolved in distilled water with the help of Polyoxyethylene 20 sorbitan mono laurate (Tween-20) in such a way that 0.3 ml of final preparation contained 300 μg of the compound. The sample was administered to the rats of group B intraperitoneally at a dose 300 μg/rat/day for 21 consecutive days.

Gross general observation after drug administration: The rats were observed daily to note the following features: behaviour, CNS excitation, CNS depression, food intake, salivation, diarrhoea and muscular weakness.

The body weight of each rat of group A and B were measured before administration of drug and after completion of the treatment, prior to sacrificing the animals.

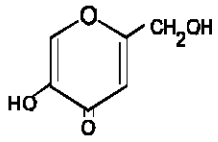
Study of hematological profiles, biochemical parameters of blood and histopathology of liver, kidney, lung, heart and spleen: For hematological studies, blood was drawn from the tail vein of each rat of group A and B before drug administration. Blood smears were made on glass slides and stained with Leishmen reagent to perform TC, DC and platelet count. Blood was drawn from each rat to estimate the hemoglobin percentage by a hemocytometer. The tests were repeated on the 7^{th}, 14^{th}, and 21^{st} day after the compound administration.

For the determination of SGOT (Serum glutamate-oxaloacetate transaminase), SGPT (Serum glutamate –pyruvate transaminase), serum alkaline phosphatase, urea, uric acid and creatinine, blood samples were collected separately from each of the control and experimental rat from their throat vein after sacrificing at the end of 21 days 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 and Coulombe and Avreau, 1963).

For histopathological studies of liver, kidney, heart, lung and spleen, 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 the Bangladesh Sericulture Research Institute, Rajshahi, Bangladesh.

Results and Discussion

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



[5-hydroxy-2(hydroxy methyl)-4H-pyran-4-one]

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

Group	Dose (i.p.) μg/rat/day	Body weight (gm) before drug treatment n=5, $m_1 \pm SD_1$	Body weight (gm) after drug treatment n=5, $m_2 \pm SD_2$	% change	Calculated t value	t value at 5% level of significance	Remark
A	300 μl vehicle	111.3±3.407	112±4.882	+1.168	- 0.488	2.306	NS
B	300 μg compound	116.2±3.31	116.4±2.653	+0.172	+0.105	2.306	NS

m_1 and m_2 = Sample mean value SD_1 and SD_2 = Standard deviations of control and experimental group respectively.
n = Number of rats, + = Increase, NS = Non significant.

Table 2: Hematological profiles of rats of group- A (control, treated with vehicle)

Hematological parameters	Normal rats	Rats treated with vehicle		
	1st day	7th day	14th day	21st day
(I) Total RBC count (million/cu.mm)	5.0	4.8	4.5	3.9
	5.1	5.2	4.3	3.6
	4.4	4.4	5.0	4.8
	5.0	4.2	4.8	4.3
	4.9	4.6	5.0	3.7
	4.88±0.24	4.72±0.38	4.72±0.35	3.73±0.12
(ii) Total WBC count	12.30	12.00	12.00	11.70
(Thousand/cu.mm)	12.00	11.80	11.40	11.60
	11.50	11.40	10.70	12.20
	11.80	11.50	10.80	11.40
	11.50	12.00	11.50	11.30
	11.82±0.43	11.74±0.28	11.51±0.59	11.60±0.17
(iii) Differential count of WBC				
a. Neutrophil	36	38	37	36
	38	34	35	36
	38	36	34	33
	35	33	37	37
	38	35	32	30
	37±1.26	35.2±1.72	35±1.89	34.4±2.57
b. Lymphocyte	52	51	51	51
	53	54	53	51
	51	54	52	58
	57	57	54	54
	53	58	57	59
	53.2±2.03	54.8±2.48	53.4±2.05	54.6±3.38
c. Monocyte	7	5	6	7
	5	6	7	6
	6	5	7	4
	4	5	5	5
	3	4	6	5
	4.8±1.41	5±0.63	6.2±0.074	5.2±1.16
d. Eosinophil	5	6	6	6
	4	6	5	7
	5	5	7	5
	4	5	4	4
	6	3	5	4
	3.41±0.23	5.0±0.63	5.4±1.01	5.2±1.16
(iv) Platelet count (million/cu.mm)	3.40	3.55	3.40	3.00
	3.25	3.10	3.50	3.80
	3.20	3.20	3.50	3.70
	3.80	3.00	3.20	3.30
	3.70	3.40	3.10	3.40
	13.14±0.53	3.25±0.20	3.35±0.17	3.39±0.24
(v) Hemoglobin (%)	13.3	13.9	14.0	12.9
	12.8	12.9	13.8	13.3
	13.5	13.0	13.3	13.6
	12.3	13.5	13.0	13.7
	13.8	13.0	13.2	13.5
	13.14±0.53	13.26±0.38	13.46±0.37	13.4±0.28

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

Hematological profiles: The hematological profiles of the experimental rats were studied after intraperitoneal administration of the compound to check the hematological disorders. No abnormalities were found in total count of WBC & RBC, differential count of WBC, platelet count and haemoglobin percentage of the drug treated rats in comparison to control rats (Table 2&3).

Biochemical parameters of blood: Biochemical parameters of blood, e.g. SGOT, SGPT, SALP (Serum alkaline phosphatase), urea, uric acid and cretonne of both, experimental and control rats were determined to check any change of these parameters due to the administration of compound with respect to control rats (Table-4). It was found that most of the parameters were slightly increased with respect to control but remained within the normal range.
From the Table 4, it was found that the changes are also statistically insignificant. These results indicated that the

Table 3: Hematological profiles of rats of group- B (Treated with the compound)

Hematological parameters	Normal rats	Rats treated with the compound		
	1st day	7th day	14th day	21st day
(I) Total RBC count	4.6	4.0	4.1	4.0
(million/cu.mm)	4.8	3.8	4.9	3.9
	4.0	4.8	4.7	4.7
	4.2	4.7	4.1	4.5
	4.0	4.2	4.2	4.4
	4.32 ± 0.32	4.3±0.32	4.4±0.33	4.3±0.30
(ii) Total WBC count	11.20	12.00	11.50	12.00
(thousand/cu.mm)	11.60	11.70	11.70	11.00
	11.80	11.70	10.30	12.20
	12.10	11.40	11.20	11.70
	11.70	12.00	10.00	10.90
	11.68±0.2	11.76±0.22	11.14±0.48	11.64±0.45
(iii) Differential count of WBC				
a. Neutrophil	39	40	41	39
	38	39	43	42
	40	39	40	41
	42	41	39	40
	41	42	39	38
	40 ± 1.44	40.2±1.16	40.4±1.49	40.0±1.41
b. Lymphocyte	50	48	50	52
	51	50	50	47
	51	53	51	53
	50	51	53	51
	50	50	51	52
	50.4 ± 0.48	50.4±1.62	51 ±1.09	51 ±2.09
c. Monocyte	6	6	4	6
	5	6	4	6
	5	5	5	3
	4	4	4	5
	6	4	5	5
	5.2 ± 0.74	5.0±0.89	4.4±0.48	5±1.09
d. Eosinophil	5	6	5	3
	6	5	3	5
	4	3	4	3
	4	4	4	4
	3	4	5	5
	3.6 ± 0.8	4.4±0.48	4.2±0.74	4.0±0.89
(iv) Platelet count	3.40	3.40	3.00	3.55
(million/cu.mm)	3.80	3.55	3.10	3.10
	3.70	3.50	3.30	3.20
	3.25	3.20	3.90	3.00
	3,20	3.10	3.30	3.40
	3.47 ± 0.24	3.35±0.17	3.12±0.16	3.25±0.20
(v) Hemoglobin (%)	12.8	13.9	13.3	12.09
	13.0	12.7	13.0	13.4
	13.5	13.0	13.2	13.5
	13.0	13.5	13.0	13.8
	13.3	13.1	12.8	12.8
	13.12 ± 0.24	13.2±0.41	13.12±0.12	13.11±0.60

Table 4: Effect of the compound on biochemical parameters of rat's blood i.p. Administration of 300 μg/rat/day for 21 consecutive days

Biochemical parameters	Control rats (group A) n=5, $M_1 \pm SD_1$	Experimental rats (group B) n =5, $M_2 \pm SD_2$	% change	Calculated t value	t value at 5% level of significance	Remark
SGOT (IU/L)	11.4 ± 1.019	12.2 ± 1.469	+0.8	+1.00	2.306	NS
SGPT (IU/L)	9.2 ± 0.718	9.2 ± 1.326	0	0	2.306	NS
SALP (IU/L)	402 ± 1.732	40.27 ±1.482	+0.675	+0.264	2.306	NS
Serum Bilirubin (m mol/l)	6.94 ± 1.036	6.96 ± 0.801	+0.288	+0.034	2.306	NS
Creatinine (mg%)	8.42 ± 0.44	8.48 ± 0.279	+0.06	+0.257	2.306	NS
Uric acid (mg.%)	7.42 ± 0.43	7.56 ± 0.546	1.886	+0.451	2.306	NS
Urea (m mol/L)	3.14 ± 0.224	3.2 ± 0.209	+1.91	+0.437	2.306	NS

M_1 and M_2 = Sample mean value, SD_1 and SD_2 = Standard deviation. n = Number of rats, + = Increase, NS = Non significant

Table 5: Effect of compound on histopathology of rat's kidney, heart, lung, liver and spleen tissue i.p. administration of 300 μg/rat/day for 21 consecutive days.

Group	Dose (i.p)	Histopathological changes observed				
		Heart	Kidney	Liver	Lung	Spleen
A	300 μl/rat/day (Vehicle)	NAD	NAD	NAD	NAD	NAD
B	300 μg/rat/day (Compound)	NAD	NAD	NAD	NAD	NAD

NAD = No abnormality, detected.

compound has no adverse effects on liver and kidney functioning.

Histopathological studies: Histopathological studies of liver, kidney, lung and spleen of both control and experimental rats were performed for 21 consecutive days (Table-5). No detectable difference in the histopathology of these organs of control and drug treated rats were observed when viewed under oil immersion objective. This indicates that the compound has no effect on cellular structures, i.e. the compound does not cause degeneration of cells of these organs.

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