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Sub-acute Toxicity Studies of a Metabolite of *Streptomyces* Species

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Abstract: The ethyl acetate extract of yeast extract-glucose broth medium of an Actinomycetes strain, *Streptomyces* yielded a reddish yellow metabolite by chromatographic technique and was identified as 2-N-butanamide-3-methyl-4-methoxy-5-β-L-arabinosyl-propanophenone (MZ-4) on the basis of spectral data. The sub-acute toxicity study of this metabolite was carried out on long Evan's rats. The change of haematological and biochemical parameters were statistically insignificant. No abnormalities were found in the histopathology of the liver, kidneys, heart, lungs and spleen in the experimental group of rats at a dose of 300 μg rat^{-1} day^{-1} for 14 consecutive days, when compared with the control group.

Key words: Sub-acute, 2-N-butanamide-3-methyl-4-methoxy-5-β-L-arabinosyl-propanophenone, *Streptomyces*, long Evan's rats

Introduction

With regards to the number of antibiotics produced, the bacterial order actinomycetales in particular the genus of *Streptomyces* occupies the first position (Waksman and Woodruff, 1990). In recent years, owing to the indiscriminate use of antibiotics and other unknown reasons, the pathogenic organisms are gaining resistance to existing antimicrobial agents. Hence, the search for the isolation of new, more potent and safer antimicrobial compounds are going on and at the same time many of the marketed antibiotics are being withdrawn due to the serious side effects (Roche, 1985). With this concept, a strain of Actinomyces was isolated from a soil sample of Bogra, a northern district of Bangladesh and was identified as *Streptomyces* species (Holt *et al.*, 1994). To yield maximum metabolites, the organism was grown on yeast extract glucose broth media at 37.5°C. The filtrated liquid broth was extracted with ethyl acetate. By using thin layer chromatographic technique (Touchston and Dobbins, 1978), a pure antimicrobial compound (MZ-4, Fig. 1) was isolated from the ethyl acetate extract and was identified as 2-N-butanamide-3-methyl-4-methoxy-5-β-L-arabinosyl-propanophenone by spectral analysis (Sultan, 2002).

Since almost all of the drugs show unavoidable toxic effects, therefore, in order to assess the safety and efficacy of a drug, toxicity studies are carried out in animals like mice, rats, guineapigs, dogs and monkeys etc. Previously we reported toxicity studies of a number of antimicrobial metabolites isolated from plants and microbes (Rahman *et al.*, 2000; Rahman *et al.*, 2001; Choudury *et al.*,

2001). In continuation of our research works, we isolated an active metabolite 2-N-butanamide-3-methyl-4-methoxy-5-β-L-arabinosyl-propanophenone from a *Streptomyces* species and it's antibacterial activity was conducted (Sultan *et al.*, 2002).

Herein we wish to report the toxicity study of the compound, 2-N-butanamide-3-methyl-4-methoxy-5-β-L-arabinosyl-propanophenone at a dose of 300 µg rat^{-1} day^{-1} on long Evan's rats for 14 consecutive days.

Materials and Methods

Isolation of antibiotic: The experiment was carried out in Microbiology Research Laboratory, Department of Pharmacy, Rajshahi University, Bangladesh during January-September, 2001. A strain of Actinomyces was isolated from a soil sample of Bogra, a northern district of Bangladesh and was identified as *Streptomyces* species (Holt *et al.*, 1994). To yield maximum metabolites, the organism was grown on yeast extract glucose broth media at 37.5°C. The filtrated liquid broth was extracted with ethyl acetate. By using thin layer chromatographic technique (Touchston and Dobbins, 1978), a pure antimicrobial compound (MZ-4, Fig. 1) was isolated from the ethyl acetate extract and was identified as 2-N-butanamide-3-methyl-4-methoxy-5-β-L-arabinosyl-propanophenone by spectral analysis (Sultan, 2002).

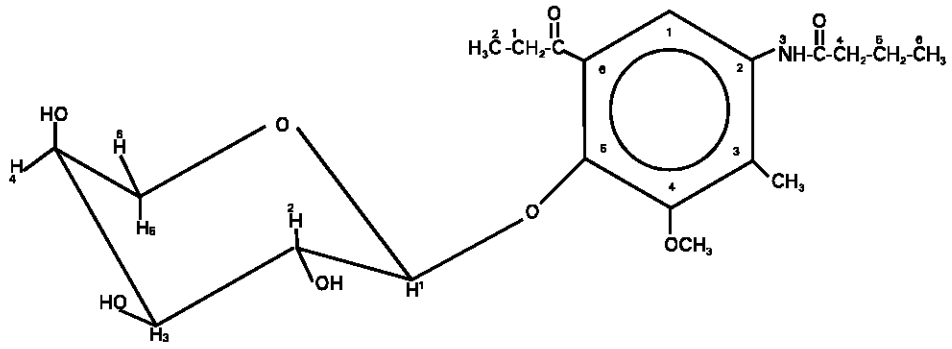

Fig. 1: 2-N-butanamide-3-methyl 4-methoxy-5-β-L-arabinosyl- propanophenone

Collection, maintenance and grouping of experimental rats: For the purpose of study, 8 long Evan's rats of same sex (male) and age (adult) were collected from ICDDRB, Mohakhali, Dhaka. The rats were kept in properly numbered iron cages individually in a clean animal house with an optimal room temperature (25-30°C) and were given ideal food (Hawk *et al.*, 1954). The animals were maintained in this way for 15 days before drug administration and continued up to the end of the experiment. Weight of the individual rat was taken and these were grouped into two groups. The rats of group B (average weight 117.5 g) were used for experiment while that of group A (average weight 103.75 g) were used as control.

Sample administration: The antibiotic (MZ-4) was dissolved in distilled water using tween-20 as co-solvent, so that 0.3 ml contained 300 µg of the antibiotic. The rats in group A and B were injected intra-peritoneally with vehicle (300 µl isotonic saline) and antibiotic (MZ-4), 300 µg rat^{-1} day^{-1} respectively.

Gross general observation after MZ-4 administration: During the whole experimental period their behaviour, central nervous system (CNS) excitation, CNS depression, reflexes, muscular weakness, salivation, diarrhea and food intake were observed. The body weight of each rat of group A and B were measured before administration of the drug and after the completion of the treatment prior to scarify them.

Haematological profiles of blood: For haematological studies, blood was drawn from the tail veins of all the rats in group A and B before the commencement of drug administration. Then blood smears were made on glass slides and stained with "Leishmen reagent" to perform total count (TC), differential count (DC) and platelet count. With the use of capillary tubes blood was drawn from each of the rat to estimate the hemoglobin percentage by a haemocytometer. This was the pre-haematological study on normal rats. Post haematological studies were done on 7 and 14 days after the commencement of drug administration following the same procedure used for normal rats.

Biochemical parameters of blood: For the study of biochemical parameters, the rats of all the groups were sacrificed with the help of a surgical blade No. 22 on the 14th day of treatment with MZ-4 and the blood were collected in plastic centrifuge tubes. These were then allowed to clot at 40^{0}C for 4 h. After clotting, the blood samples were centrifuged at 4000 rpm for 15 min using a WIFUNG centrifuge LABO-50M. The clear straw colour serum was then collected in vials with Pasteur pipette and stored at -20^{0}C. Then the enzymes SGOT, SGPT, serum alkaline phosphatase and serum creatinine, serum bilirubin, uric acid and urea were determined by using the procedures of Reitman and Frankel (1957), Fawcett and Scott (1960) and Coulombe and Favreau (1963).

Histopathological study of liver, kidneys, heart, lungs and spleen: The liver, kidneys, heart, lungs and spleen of all of the rats of group A and B were collected after sacrificing them at 14th day of observation. The tissues were sliced into pieces and immersed in 10% formalin for three days, processed, stained with "Harris Hematoxilin and eosin reagent", mounted on glass slides with diphenyl xyline mounting fluid and observed under microscope.

Results and Discussion

Gross general observation: The rats of group A and B were being treated with vehicle and MZ-4 respectively showed no signs of tremor, convulsions and reflex abnormalities. The body weights of all the rats (both group A and B) were increased after treatment (Table 1). Moreover, no muscular

numbness of the hind and fore legs, salivation or diarrhea was observed. The food intake per day was also found normal. So, from the results, it is decided that drug has no effect on normal growth.

Haematological profiles: Haematological profiles were studied on normal rats (before treatment) and after 7 and 14 days of treatment. Each time the value of the parameters in each rat was changed slightly. However, the parameters remained within the normal range (Tables 2, 3).

Biochemical parameters: Biochemical parameters were studied on normal rats (before treatment) and after 7 and 14 days of treatment, however the parameters remain within the normal range. This indicates that the antibiotic has no adverse effects on liver and kidney function (Table 4).

Histopathological studies: At the 14th day of drug treatment and observation, the animals of both control and experimental groups were sacrificed and the organs like liver, kidneys, lung, spleen and heart were isolated and histopathological examinations were done. No detectable abnormality was observed between the control and the drug treated rates, when the tissue slides were examined under microscope. This indicates that the compound has no effects on cellular structure, i.e., the compound does not cause degeneration of cells of these organs (Table 5).

Discussion

As a part of our continuous search for novel microbial metabolite, we isolated the active metabolite 4-hydroxy nitrobenzene and its significant antimicrobial screening was reported (Zakir *et al.*, 2002). The present work is the continuation of this effective antimicrobial screening. The aim of this study was to evaluate the safety margin of the antibiotic prior to clinical trial, as all drugs are toxic at higher doses (Goldstein, 1974). Moreover, even at therapeutic blood level some drugs have unavoidable side effect.

The result of this study demonstrate that although the isolated active metabolite possesses slight change of haematological and biochemical parameters (at a dose of 300 µg rat^{-1} day^{-1} for 14 consecutive days) but these changes were statistically non-significant. No abnormalities were found in the histopathology of the liver, kidneys, heart, lung and spleen in the experimental group. Thus the antibiotic may be considered as a safe and effective antimicrobial agent. The findings of this and previous investigation (Rahman *et al.*, 2002) would give valuable support to make clinical

Table 1: Effect of antibiotic (MZ-4, 300 µg rat^{-1} day^{-1}) on body weight of rats (n= 4)

Groups	Dose (Intra-peritoneally) µg rat^{-1} day^{-1}	Body weight (gm) before drug treatment $M_1 \pm SD_1$	Body weight (g) after drug treatment $M_2 \pm SD_2$	% change	Calculated t-value
A	300 µl vehicle	103.75±3.96	109.75±3.56	+5.78	+2.26 NS
B	300 µg comp.	117.50±5.41	122.75±6.18	+4.47	+0.62 NS

M_1 and M_2 = Sample mean value, SD_1 and SD_2 = Standard deviations, n = Number of rats, + = Increase, NS =Non significant, Group A= Control rats, Group B= Experimental rats, t-values at 5% level of significance 2.447

Table 2: Hematological profiles of group-A rats

Hematological parameters	Normal rats 1st day	Rats treated with vehicle 7th day	Rats treated with vehicle 14th day
Total RBC count (million/cu.mm)	4.80±0.07	5.25±0.11	5.075±0.083
Total WBC count (No./cu. mm)	7,225±108.97	7150±111.80	7325±108.97
Differential count of WBC			
Neutrophil	42.5±2.06	44.25±1.64	42.625±2.0
Lymphocyte	51.0±2.35	51.25±0.83	52.25±1.79
Monocyte	5.0±1.58	3.25±1.29	3.25±0.83
Eosinophil	1.5±0.5	1.25±0.43	1.75±0.43
Platelet count No./cu. mm)	322500±15618.4	332500±19202	310000±18708
Hemoglobin (%)	13.65±0.25	13.775±0.109	13.32±2.21

RBC= Red blood cells, WBC= White blood cells

Table 3: Hematological profiles of group-B rats

Hematological parameters	Normal rats 1st day	Rats treated with antibiotic MZ-4 7th day	Rats treated with antibiotic MZ-4 14th day
Total RBC count (million/cu.mm)	4.85±0.18	5.05±0.112	4.925 ± 0.083
Total WBC count (No./cu. mm)	8350±415.33	8475±396.07	8425± 402.34
Differential count of WBC			
Neutrophil	35±1.87	36.5±1.66	35.75 ±. 1.09
Lymphocyte	53±1.58	55.5±1.12	56.75 ± 1.3
Monocyte	9.25±0.83	6.75±0.83	6 ± 1.0
Eosinophil	2.75±1.43	1.25±0.43	1.5±0..5
Platelet count (No./cu. mm)	326250±16345.87	336250±12930.10	328000±16792.86
Hemoglobin (%)	9.325±0.61	8.9±0.64	9.175±0.55

Table 4: Effect of antibiotic (MZ-4) on rats blood after i.p. administration of 300 µg rat^{-1} day^{-1} for 14 consecutive days (n=4)

Biochemical parameters	Group A, $M_1 \pm SD_1$	Group B, $M_2 \pm SD_2$	% change	Calculated t-value
SGOT (IU L^{-1})	10.825±0.72	11.25±1.30	+3.92	0.572NS
SGPT (IU L^{-1})	8.5±0.5	9.25±0.83	+8.82	1.55NS
SALP (IU L^{-1})	44.25±2.86	45.25±2.59	+2.26	0.518NS
Serum uric acid (mg dl^{-1})	7.3±0.369	7.05±0.11	-3.424	1.302NS
Urea (mg dl^{-1})	18.25±0.83	18.75±1.09	+2.74	0.685NS
Serum creatinine (mg dl^{-1})	0.85±0.09	0.9375±0.065	+10.29	1.576NS
Serum bilirubin (mg dl^{-1})	0.28±0.014	0.29±0.0122	+3.57	1.075NS

SGOT= Serum glutamate oxaloacetate transaminase, SGPT = Serum glutamate pyruvate transaminase, SALP= Serum alkaline phosphatase, M_1 and M_2 = Sample mean value, SD_1 and SD_2 = Standard deviations, n = Number of rats, + = Increase, NS =Non significant, Group A= Control rats, Group B= Experimental rats, t-values at 5% level of significance 2.447

Table 5: Histopathological studies after treatment with antibiotic MZ-4 at a dose level of 300 µg rat^{-1} day^{-1} for 14 consecutive days

		Histopathological changes			
Group of	Dose (i.p.)	Liver	Heart	Lungs	Kidney
A	300 µl (vehicle)	NAD	NAD	NAD	NAD
B	300 µg (MZ-4)	NAD	NAD	NAD	NAD

NB: NAD= No abnormality detected, Group A= Control rats, Group B= Experimental rats

trial of the isolated metabolites to gate a more potent antibiotic that would be helpful for human being.

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