

**Original Article****Toxicological studies of a metabolite of *Streptomyces* species on Brine shrimp and Mice**

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**Abstract**

The cytotoxic activity of the crude chloroform extract of *Streptomyces* species and a pure compound, 4-hydroxy nitrobenzene (ZS-3) isolated from chloroform extract was determined by brine shrimp lethality bioassay. The LD<sub>50</sub> values of chloroform extract and compound (ZS-3) and ampicillin trihydrate were found to be 5.6, 6.6 and 5.0 µg ml<sup>-1</sup>, respectively. The acute toxicity of the compound (ZS-3) was also performed on *Swiss albino* mice. The median acute toxicity value (LD<sub>50</sub>) of ZS-3 was found to be 62 mg kg<sup>-1</sup>. Thus the isolated compound ZS-3 seems to be safe for therapeutic use.

TAJ 2010; 23(1): 6-9

**Introduction**

The concept of antibiosis opened a new field of research for the isolation of antibiotics from microorganisms and so far more than 4,000 such antibiotics are known. In the United States and Japan between 1953 to 1970 approximately 85% of the antibiotics are produced by *Actinomycetes*, 11% by fungi and 4% by bacteria (Reiner, 1982). Among the *Actinomycetes*, most of the research work has been carried out on *Streptomyces* species to search for antibiotics. Based on this concept, the field for research of newer antibiotics was broadened. As a part of our continuing search for microbial metabolites from soil samples, soil samples were collected from the different parts of Bangladesh and a strain having antimicrobial activity was isolated from the soil of Meherchandi (Near to fine and arts department of Rajshahi University) Rajshahi, Bangladesh and was identified as *Streptomyces* species (Sathi *et al.*,

2001 and Holt *et al.* 1994). From the chloroform extract of the yeast-extract glucose broth culture filtrate of the organism, an antimicrobial agent was isolated by preparative thin layer chromatographic technique (PTLC) (Sathi *et al.*, 2002 and Egon and Stahl, 1969) and was identified as 4-hydroxy nitrobenzene (ZS-3) by spectral analysis (Sathi *et al.*, 2002). The cytotoxicity by brine shrimp lethality bioassay (Rahman *et al.*, 2007, Mbwanbo *et al.*, 2007 and Mayer *et al.*, 1982) is an excellent development in the bioassay for the discovery of the bioactive natural products. Here the simple zoological organism (Brine Shrimp nauplii) is used as a convenient monitor for screening. Acute toxicities (Ogbonnia *et al.*, 2009 and Goldstein, 1974) are done to estimate the nature and extent of acute toxicity and serious adverse side effects that may follow the administration of the compound. We, herein, report the cytotoxic activity of both extract and the compound ZS-3 and acute toxicity of the compound ZS-3.

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## Material and Methods

### *Collection of organism:*

The organism was isolated from the soil sample, collected from Meherchandi (Near fine and arts department, Rajshahi University), Bangladesh at the depth of 0.75 meter using “crowded plate technique”. The organism was identified as *Streptomyces* species (Sathi *et al.*, 2001), by morphological and biochemical study (Williams *et al.*, 1983).

### *Extraction, isolation and characterization of the compound:*

For the collection of metabolites, the *Streptomyces* species was grown optimally in yeast-extract glucose broth media at 37.5°C in order to get the optimum production. The liquid broth was separated through filtration. Then the filtrate was extracted with chloroform and concentrated. By the use of PTLC technique, a pure compound (ZS-3) was isolated from the chloroform extract and identified as 4-hydroxy nitrobenzene (Sathi *et al.*, 2002) on the basis of its spectral data.

### *Preparation of sea water:*

38 gm of sea salt (non-iodized NaCl) was weighed accurately and dissolved in distilled water to make a volume of 1.0 liter.

### *Hatching of Brine Shrimp:*

Sea water was kept in small tank and shrimp eggs were added in the one side of the divided tank and this side would attract hatch shrimp through perforation in the dam. Constant oxygen supply was carried out and a constant temperature was maintained. Two days were needed for the shrimps to hatch and mature as nauplii (Larvae). The hatched shrimps were attracted to the lamp on other side of the divided tank through the perforations in the dam. These nauplii was taken for bioassay.

### *Preparation of the sample solution and application to the nauplii to the vial:*

3.0 mg of each sample (crude chloroform extract; compound, ZS-3 and ampicillin trihydrate) was taken and dissolved in 0.6 ml of Dimethyl Sulfoxide (DMSO) to make the concentration of 5  $\mu\text{g ml}^{-1}$ . The experiment was done in five concentration of each sample. Each concentration contained 3 vials consisting of 10 nauplii in 5.0 ml

of sea water. The concentration of the sample in each vial was made 5, 10, 20, 40 and 80  $\mu\text{g ml}^{-1}$  respectively. The same assay procedure was carried out for the standard ampicillin trihydrate (ATH). For the control group, 3 vials, each contain 10 brine shrimp nauplii in 5.0 ml sea water and 20.0  $\mu\text{l}$  DMSO.

### *Counting of nauplii:*

After 24 hours, the vials were observed and the number of survived in each vial was counted and the results were noted. From this data, percentage of mortality of nauplii was calculated at each concentration for each sample.

### *Studies of the acute toxicity:*

During screening of a new drug, acute toxicity was done to estimate the nature and extent of acute toxicity and abrupt side effects that may follow the administration of the drug.

### *Determination of LD<sub>50</sub>:*

LD<sub>50</sub> is the dose that is likely to cause death of 50% of the test animal. It is the most common measure of acute toxicity. In the LD<sub>50</sub> determination each animal is classified as dead or alive at specified time after drug administration. LD<sub>50</sub> were calculated by usual procedure (Gilman *et al.*, 2006).

### *Collection and maintenance of mice:*

The toxicity study was carried out using thirty (30) male and female *Swiss albino* mice weighing 30 – 35 g each, were purchased from animal house of International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). Prior to the commencement of the experiment, all the mice were acclimatized to the new environmental condition for a period of one week. During the experimental period, the mice were kept in a well-ventilated animal house at room temperature of 23  $\pm$  2°C, maintaining relative humidity 50-60% and were supplied with standard pellets supplied from ICDDR, B and fresh drinking water *ad libitum*. They were kept in cages and maintained in well-ventilated room under conditions of natural light and dark cycle.

### *Grouping of mice:*

Individual weights of the mice were taken and they were grouped into five groups. Each group

consists of five mice. Group I was used as control and others were experimental.

**Preparation and administration of ZS-3 solution:** ZS-3 (75.0 mg) was dissolved in 7.5 ml of distilled water with the help of tween-20. Thus the concentration was 10 mg ml<sup>-1</sup>. The compound was administered intraperitoneally to each of the experimental mice according to the experimental schedule. Mice of the group I received the vehicle only and used as control.

#### Data analysis:

Data were analyzed by Prism (Graph Pad Software, San Diego, CA, USA) and represented as mean ± S.E.M.

#### Results

##### *Cytotoxic effect of the chloroform extract and the compound ZS-3:*

Bioactive compounds are almost always toxic in high dose. The bioactivity of the natural product, extracts and pure compounds are detected by the brine shrimp lethality bioassay, a bench top bioassay method for cytotoxic effects. The chloroform extract and the compound ZS-3 showed positive results indicating that both compounds and extracts were cytotoxic. Mortality rate of brine shrimp nauplii was found to be increased with the increase of concentration of the samples and the logarithm of concentration versus percent of mortality was plotted and a best fitted line was drawn which showed an almost linear correlation. From the graph the LC<sub>50</sub> (concentration at which 50% mortality of the nauplii occurred) was determined by extrapolation and found to be 5.6, 6.6 and 5.0 µg ml<sup>-1</sup> for chloroform extract, compound ZS-3 and ampicillin trihydrate, respectively. The results are given in the table 1.

##### *Acute toxicity studies:*

After the administration of the drug to the mice, both experimental and controlled group animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the compound, to observe any death or changes in general behavior and other physiological activities (Bürger et al 2005)

The logarithm of dosage regimen was plotted in X-axis and the mortality rates in probit unit were plotted in Y-axis. This gave a straight line. Then LD<sub>50</sub> was determined by plotting drawing a vertical line on the X-axis from the point of straight line where the probits of mortality unit 5 (50% the mortality) intercepted. The LD<sub>50</sub> value was found to be 62 mg kg<sup>-1</sup> for the compound ZS-3.

Cytotoxic action of a drug is exhibited by disturbing the fundamental mechanisms concerned with the cell growth, mitotic activity, differentiation and function (Gillman et al., 2006). Although the exact mechanism of cytotoxic action of the metabolites and the compound ZS-3 could not be explained by this preliminary test, but the compound ZS-3 may be used as safe and effective chemotherapeutic agent.

From the value of LD<sub>50</sub>, it can be concluded that the drugs can be used as higher doses. There was no mortality in control group. So, it was concluded that the compound was biologically active.

#### Acknowledgements

The authors wish to thank Dr. Naoki Sugimoto, National Institute of Health Sciences, Tokyo, Japan for spectral analysis.

**Table 1:** Results of the brine shrimp lethality bioassay of the chloroform extract, compound ZS-3 and the standard, ampicillin trihydrate.

Test sample	Concentration (C) µg ml <sup>-1</sup>	% of mortality*	Log (C), µg ml <sup>-1</sup> from the graph
Control	0	0	
Chloroform extract	5	38.2 ± 1.5	5.6
	10	46.6 ± 1.0	
	20	47.0 ± 1.3	
	40	48.4 ± 1.4	
	80	56.7 ± 1.2	
ZS-3	5	48.4 ± 2.0	6.6
	10	50.0 ± 1.87	
	20	58.2 ± 1.6	
	40	69.3 ± 0.8	
	80	77.5 ± 1.0	
Ampicillin trihydrate	5	47.3 ± 1.3	5.0
	10	52.3 ± 1.4	
	20	60.0 ± 1.2	
	40	71.2 ± 0.8	
	80	89.5 ± 1.3	

\*Each experiment was performed in triplicate and the results are expressed as the mean ± S.E.M.

Table 2: The acute toxicity of the compound ZS-3 from *Streptomyces* species in mice

Group	Dose, C (mg)	Log C	Probits of mortality	LD <sub>50</sub> (mg Kg <sup>-1</sup> )
I	200 µl Vehicle	-	-	
II	25	1.39	3.5 ± 0.2	62
III	50	1.69	4.9 ± 0.1	
IV	100	2	7.5 ± 0.2	
V	200	2.31	8.9 ± 0.3	

Data are expressed as mean ± SEM, n=5.

## References

- Bürger C, Fischer DR, Cordenunzi DA, Batschauer de Borba AP, Filho VC, Soaresdos Santos AR. Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasiliensis*) (Asteraceae) in mice. *J. Pharm. Sci.* (www.cspCanada.org) 2005; 8(2): 370-373.
- Egon and Stahl. *Thin Layer Chromatography- A laboratory Hand Book*. Revised and extended second version, Springer Verlag, New York, USA. 1969.
- Gillman GA, Goodman LS, Rall TW, Murad F. *The pharmacological basis of therapeutics*. 11<sup>th</sup> Edition. Macmillan Publishing Co. New York. 2006; 25-27.
- Goldstein AL, Aronow L and Kalkan SM. *Principles of drug action*. Second edition. Wiley Biomedical Health Publication. 1974:376-381
- Holt JG, Kreig NR and Sneath HA. *Bergey's Manual of Determinative Bacteriology*. 9<sup>th</sup> edition. 1994:376-381.
- Mbwanbo ZH, Moshi MJ, Mosimba PJ, Kapingu MC and Nondo RS. Antimicrobial activity and brine shrimp toxicity of extract of *Terminalia brownii* roots and stem. *BMC Complementary and Alt. Med.* 2007; 7:1-5.
- Mclaughlin JL. Bioassays for discovery of antitumor and antiviral agent from natural sources, *Proceedings of NIH workshop vol. 18*, Bethesda (1988):220.
- Meyer BN, Ferringni NR, Putnam JE, Jacobsen LB, Nichols DE and Mclaughlin JL. Brine shrimp: a convenient bioassay for active plant constituents. *Planta Med.*1982; 4: 31-34.
- Ogbonnia SO, Olayemi SO, Anyika EN, Enwuru VN and Poluyi OO. Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of *Parinari curatellifolia* Planch (*Chrysobalanaceae*) seeds in rats. *Afr. J. Biotech.* 2009; 8 (9):1800-1806.
- Reiner R. *Antibiotics: An introduction*, Roche Scientific Service. Switzerland.1982: 21-25.
- Rahman MM, Alam AHMK, Sadik G, Islam MR, Khondokar P, Hossain MA, Rashid MA. Antimicrobial and cytotoxic activities of *Achrenthes ferruginea*. *Fototherapia.* 2007;7:260-262.
- Sathi ZS, Rahman MAA, Gafur MA. Identification and in vitro antimicrobial activity of a compound isolated from *Streptomyces* species. *Pak J Biol Sci.* 2001; 4 (12): 1523-1525.
- Sathi ZS, Sugimoto N, Khalil MI, Gafur MA. Isolation of a yellowish antibiotic pigment 4-hydroxy nitrobenzene from a strain of *Streptomyces*. *Pak J Biol Sci.* 2002; 52:201-203.
1. Williams ST, Goodfellow M, Wellington EMH, Vickers JC, Alderson G, Sneath PHA, Sackin MJ and Mortimer AM. A Probability Matrix for Identification of some *Streptomyces*. *J Gen Microbiol* 1983; 129: 1815-1830.

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