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Aquatic toxicity of dimethyl sulphoxide on the Indian Major Carp, *Catla catla* (Hamilton)

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Abstract: DMSO is frequently used as carrier solvent in toxicological experiments due to its exceptionally low toxicity and environmental impact and also to achieve more effective dispersion of the toxicants. Aquatic toxicity studies of DMSO on the respiratory rates and the biochemical constituents of muscle and liver in catla revealed minimal lethality and negligible effect on the physiology of the test organism. This study helps in the choice of percentage of the carrier solvent that can be used in the preparation of toxicants for any toxicological studies involving fish.

Key words: Aquatic toxicity - dimethyl sulphoxide - *Catla catla*

Introduction

In aquatic toxicological studies involving biological organisms, the effect of the principal toxicant is the most crucial aspect. Some of the toxicants require an additive or a carrier solvent to make it miscible in the medium due to poor water solubility of chemicals and this could result in an independent effect of stress on the organism by such solvents. In this scenario, the present study helps in assessing the effects of such solvents exerted on the test organism.

Dimethyl sulphoxide (DMSO) has received considerable attention because of its remarkable biological properties: it passes rapidly through many animal membranes, enhances absorption of a wide variety of substances and is also a versatile solvent with little systemic toxicity when given to animals. The membrane-penetrating ability of dimethyl sulfoxide may enhance diffusion of other substances through the skin.

DMSO is an important solvent for small molecule studies as it provides a nearly universal approach for the solubilisation of small molecules. Because of its physicochemical properties, high solvent power, low chemical reactivity and relatively low toxicity, DMSO has become the solvent of choice (Johannesson *et al*., 1997) for such studies.

DMSO is the most common solvent carrier used in aquatic toxicity tests to prepare miscible concentrations of the chemical toxicants in order to help achieve more effective dispersion of the toxicants (Hutchinson *et al*., 2006). Moreover, the more compelling attributes for the selection of DMSO are its exceptionally low toxicity and environmental impact (Mortensen & Arukwe, 2006). Hence it is imperative to know the extent of effect of DMSO on the organisms.

The present study is an attempt to investigate the effect of the DMSO on the respiratory rates and the biochemical components (Total sugars, Total proteins and Total lipids) of muscle and liver tissues of one of the commercially important and widely cultured Indian Major Carps, *Catla catla.*

Materials and methods

Catla fingerlings of the same size (4.5 – 5.0 cm in length and 6.2 – 6.8 g in weight) were procured from private culture ponds and brought to the laboratory in oxygen packs. The fish were acclimatized and maintained in ferro-cement tanks (3'L x 2'W x 2'H) filled with bore well water. The stock fish were fed with pelleted feed prepared with tapioca powder, groundnut oil cake, rice bran and mineral mixture (Omprakasam & Manohar, 1991) at 5% body weight in two split doses. Feeding was stopped 24 hr prior to experimentation.

Apparently healthy fish were selected for experiments and maintained in disinfected glass aquarium tanks (2'L x 1'W x 1'H) filled with water at the rate of 2 litres per fish. Four experimental groups were maintained with aqueous solutions of DMSO (EMerck-AR grade) at the rate of 0.5%, 1.0%, 1.5 % and 2.0% (v/v), along with suitable controls without the toxicant.

Observations were made for structural behavioral and internal pathological conditions. Ten fish from control as well as experimental groups were sacrificed for the study of selected parameters on Day Zero, 4^{th} day and 7^{th} day of experimentation. Standard protocols were followed for the analyses.

Rate of oxygen consumption was estimated by titrimetric method following modified Winkler's method (Anon, 1984)

For the analyses of biochemical parameters, muscle and liver tissues were dissected out from the control and experimental fish after the analysis of respiratory rate.

Colorimetric method was followed for the biochemical analyses using Spectronic-21 (Bausche & Lomb) spectrophotometer. Total sugars was estimated by anthrone method (Carrol *et al*., 1956) and the total protein content in the tissues was done by folin phenol method (Lowry *et* *al*., 1951), while for the estimation of total lipids, the method of Bligh and Dyer (Jayaraman, 1988) was followed.

Table 1. Effect of Dimethyl sulphoxide on *Catla catla*: Oxygen Consumption (ml/g/hr)

Conc.	Zero Day	4th Day Control	4th Day Treated	7th Day Control	7th Day Treated
0.5%	0.08 ± 0.02	0.199 ± 0.010	0.355 ± 0.02	0.21 ± 0.02	0.495 ± 0.02
1.0%	0.08 ± 0.02	0.199 ± 0.010	0.485 ± 0.01	0.21 ± 0.02	0.391 ± 0.04
1.5%	0.08 ± 0.02	0.199 ± 0.010	0.213 ± 0.010	0.21 ± 0.02	0.375 ± 0.01
2.0%	0.08 ± 0.02	0.199 ± 0.010	0.290 ± 0.01	0.21 ± 0.02	0.273 ± 0.01

The results were tabulated with means of ten values and expressed as Mean ± SEM for all the conditions and parameters. Students't' test was applied to assess the statistical significance between two means.

Results and discussion

Catla fingerlings exposed to DMSO were found to be lethargic and did not respond to stimuli. Swimming activity was incoherent with occasional darting and whirling movement. Frequent surfacing and gasping were also prominently observed. These symptoms of abnormal behavior were clearly evident only for a few hours after exposure to the toxicant and regained normalcy within 24 hrs, indicating the initial adaptive response of the test fish to stress imposed by the toxicant. This shows that the toxicant DMSO is least toxic in nature and unable to evoke any lethal effect as observed by Willford (1988) in trouts and bluegills. This is confirmed by the fact that there is no mortality among the test population in any of the concentrations. The internal visceral organization of the fish also did not reveal any symptoms of poisoning or degeneration up to 7th day of exposure even at higher concentration of 2% DMSO.

In the absence of any direct evidences of effects of DMSO in fish, the conditions obtained in the present investigation are inferred based on the effects of other toxicants on the aquatic organisms.

The respiratory rate of the fish was steadily on the rise from zero day to 4th to 7th day in both the control as well as all the experimental group of fish under stress induced by DMSO (Table 1). In the treated fish, the rate of oxygen consumption was more compared to the increase noted in the untreated fish. This can be corroborated with the hyperactivity of the fish to overcome the induced stress. In aquatic organisms, the respiratory rate is an indicator of physiologic state (Yang *et al*., 2000; Santhakumar & Balaji, 2000; Bhattacharya, 2001; Nanda *et al*., 2002). Biochemical parameters of the tissues reflect the metabolic state of the fish and are influenced by pollutants and toxicants leading to death due to impaired and uncompensatory metabolic profiles.

Though there appears no documentation of the effects of DMSO, the biochemical constituents of the tissues of fishes have been shown to vary under the influence of heavy metals (Dhanapakiam & Ramasamy, 2001; Meha *et al*., 2004). Similarly the pesticides also cause severe alterations in the tissue biochemistry of fishes as evinced by (Kumar & Singh, 2000; Tilak *et al*., 2003; Mathivanan, 2004; Shrivastava & Singh, 2004).

In the present study, the fingerlings of catla showed changes in the total sugars (Table 2), total proteins (Table 3) and total lipids (Table 4) under the influence of different concentrations of DMSO on the different days of exposure. Total sugar content in the muscle and liver tissues of fish exposed to DMSO was reduced compared to their respective controls. Liver being the site of

Table 2. Effect of Dimethyl sulphoxide on *Catla catla* Total Sugars (mg/g wet wt.)

Conc. (%)	MUSCLE					LIVER				
	Zero Day	4 C	4T	7 C	7 T	Zero Day	4 C	4T	7 C	7 T
0.5	0.97 ± 0.03	1.87± 0.02	3.40± 0.05	2.75± 0.05	1.84± 0.14	26.25± 0.35	32.54± 0.79	13.58± 0.57	25.89± 2.56	23.01± 0.21
1.0	0.97± 0.03	1.87± 0.02	2.96± 0.02	2.75± 0.05	2.06± 0.15	26.25 ± 0.35	32.54± 0.79	14.39± 0.13	25.89± 2.56	24.17± 0.12
1.5	0.97± 0.03	1.87± 0.02	3.59± 0.32	2.75± 0.05	2.83± 0.07	26.25± 0.35	32.54± 0.79	24.59± 0.86	25.89± 2.56	28.13± 0.94
2.0	0.97± 0.03	1.87± 0.02	5.91± 0.17	2.75± 0.05	2.05± 0.07	26.25± 0.35	32.54± 0.79	18.11± 0.21	25.89± 2.56	24.81± 0.14

Table 3. Effect of Dimethyl sulphoxide on *Catla catla*: total proteins (mg/g wet wt.)

Conc. (%)	MUSCLE					LIVER				
	Zero Day	4 C	4T	7 C	7 T	Zero Day	4 C	4T	7 C	7 T
0.5	85.38± 0.24	46.84± 0.79	36.89± 0.70	59.25± 1.39	50.20± 1.74	52.31± 2.14	39.20± 1.33	25.50± 0.32	45.72± 2.14	24.77± 0.45
1.0	85.38 ± 0.24	46.84± 0.79	46.32± 1.23	59.25± 1.39	52.70± 1.26	52.31± 2.14	39.20± 1.33	22.15± 1.15	45.72± 2.14	29.35± 0.94
1.5	85.38 ± 0.24	46.84± 0.79	32.55± 1.42	59.25± 1.39	51.24± 1.64	52.31± 2.14	39.20± 1.33	21.42± 8.90	45.72± 2.14	25.00± 1.02
2.0	85.38 ± 0.24	46.84± 0.79	33.00± 1.81	59.25± 1.39	49.70± 0.79	52.31± 2.14	39.20± 1.33	20.36± 0.46	45.72± 2.14	28.82± 0.89

metabolism, the carbohydrates tend to accumulate for metabolic processes to occur. The fish not exposed to toxicant showed a normal trend with 26.25 mg/g on zero day, 32.54 mg/g on 4th day and reduced to 25.89 mg/g on 7th day. This reduction may be attributed to utilization of carbohydrate source available, with replenishment of sources not possible due to starvation.

Protein remains more in the muscle tissues than in the liver because of the requirement of growth factors and energy regulation needed for the swimming activity. The total protein content was depleted from 85.38 mg/g on zero day to 46.84 mg/g on 4th day control group and remained at 59.25 mg/g on 7th day. This suggests that utilization of the protein content. Compared to this, in the fish under stress due to the exposure to DMSO, the protein content was very much reduced in both the muscle and liver tissues. This suggests that the gluconeogenetic pathway has been initiated to supplement depletion of sugars by breaking down of protein to yield sugars. This becomes evident when seen together with the trend observed in the total sugar content of the tissues.

The lipids remain accumulated as reserve metabolic source to compensate for excessive loss of sugars and proteins due to impairment of physiological processes, particularly under stress. The observations made in the total lipid content of the muscle and liver tissues of the control and experimental group of fishes were in conformity to normal metabolic profiles. There is a steady accumulation of lipid from zero day to 7th day, suggesting non conversion of excess lipid to sugars by gluconeogenetic path way. This may be due to the fact that the fish were exposed to short term toxicity for 7 days only in the present study. If the study is extended for a longer duration, the lipid content also would have been utilized to compensate for the hypoglycemia and hypoprotenemia caused by the toxicant-induced stress.

The observations in this study confirm the least toxic nature of DMSO (up to 2.0% concentration) to cause any observable changes in the metabolic profile or to induce any pathological condition as an independent toxicant in the fingerlings of catla. This suggests that DMSO can be a safe solvent to carry out any toxicological studies. Moreover, majority of the chemical compounds require DMSO at 1.0% concentration in the medium to dissolve and remain stable for various studies and there appears no pathological changes in the test

Table 4. Effect of Dimethyl sulphoxide on *Catla catla*: total lipids (mg/g wet wt.)

Conc (%).	MUSCLE					LIVER				
	Zero Day	4 C	4T	7 C	7 T	Zero Day	4 C	4T	7 C	7 T
0.5	31.62 ± 0.23	37.25 ±0.19	146.36 ±0.17	72.55 ±0.15	115.45 ±0.17	55.37 ±0.57	123.59 ±0.15	199.5 ±0.26	229.61 ±0.25	245.33 ±0.29
1.0	31.62 ± 0.23	37.25 ±0.19	158.19 ±0.22	72.55 ±0.15	148.15 ±0.31	55.37 ±0.57	123.59 ±0.15	232.65 ±0.16	229.61 ±0.25	218.98 ±0.24
1.5	31.62 ± 0.23	37.25 ±0.19	127.50 ±0.23	72.55 ±0.15	130.40 ±0.25	55.37 ±0.57	123.59 ±0.15	237.50 ±0.17	229.61 ±0.25	287.50 ±0.23
2.0	31.62 ± 0.23	37.25 ±0.19	100.30 ±0.28	72.55 ±0.15	108.40 ±0.18	55.37 ±0.57	123.59 ±0.15	242.50 ±0.23	229.61 ±0.25	252.60 ±0.22

4C= 4th Day Untreated; 4T= 4th Day Treated with DMSO; 7C= 7th Day Untreated; 7T= 7th Day Treated with DMSO organism at this concentration as revealed by the present investigation.

In biological organisms, the toxic effects of the pollutants or toxicants vary depending on other biotic and abiotic factors like environmental parameters, age, dose and duration of exposure to the toxicants. Moreover, the toxic effect will be more when two or more toxicants act together in a synergistic manner (Sujatha, 2006). This study clearly indicates that DMSO is safe to use as carrier solvent in aquatic toxicity studies of chemical compounds, as it is least toxic to induce any observable pathological conditions as an independent toxicant.

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