Endosulfan Toxicity and its Reduction by Selenium: A Behavioral, Hematological and Peroxidative Stress Evaluation

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Citation

Shafiq-ur-Rehman. *Endosulfan Toxicity and its Reduction by Selenium: A Behavioral, Hematological and Peroxidative Stress Evaluation*. The Internet Journal of Toxicology. 2005 Volume 3 Number 1.

Abstract

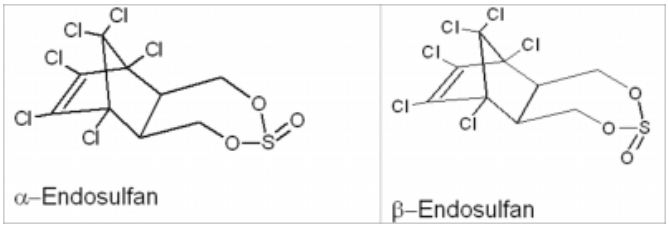
The effects of Endosulfan, a widely used organochlorine pesticide world wide, on hematology, open-field behavior including motor activity, and oxidative stress (as MDA generation) in brain were investigated in mirror carp, Cyprinus carpiovar specularis. The efficacy of Selenium was also tested to examine whether this anti-oxidant could reverse the toxic implication of Endosulfan (1 microg/L) in fish. It has been observed that almost all the tested hematological features were found to be significantly declined at 120 hr, with maximum destructions at 240 hr exposure period. Several behavioral anomalies were also detected during the exposure period. The formation of MDA, identified as an index of oxidative stress, was progressively exaggerated by Endosulfan (as seen at 120 and 240 hr). Selenium, which is an essential nutrient and a powerful anti-oxidant, was found to have protection on Endosulfan-mediated deterioration of movement behavior and aggravated lipid peroxidation in brain of fish.

INTRODUCTION

Pesticides play an important role in modern agriculture by providing dependable, persistent and relatively complete control against harmful pests with less expense and effort. They have, no doubt, increased crop yields by killing different types of pests, which are known to cause substantial or total crop damage. At the same time, these chemicals are considered as potent pollutants of the environment with undesirable effects on non-target organisms. Among pesticides, the chlorinated hydrocarbon insecticides are generally much more persistent and have long residual properties that have placed them in excessive usage in agriculture and forestry through out the world. In India alone, the agricultural consumption of endosulfan was estimated to be 5200 metric tons in 1994-95 ([1]).

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 3, 4-benzo(e) dioxathiepin-3-oxide) is a broad spectrum organochlorine insecticide of the cyclodiene group :

Figure 1



In agriculture, the widely used technical grade preparation consists of two stereoisomers, alpha- and beta-endosulfan in an approximate ratio of 70:30. The chemical is marketed by many different companies and under a variety of names including: Agrosulfan, Aginarosulfan, Endorifan, Hildan, Redsun, Seosulfan, and Thiodan. The compound is both an insecticide and acaricide, and is abundantly used in agriculture to kill insects and mites on crops, including fruits, vegetables, rice and grains, oil seeds, pulses, tea, coffee, cotton, and forestry. The pesticide kills indiscriminately, not only the pests, but also a wide range of other non-target organisms and beneficial insects with similar ramifications for species further up the food chain ([1,2,3]). Endosulfan is, therefore, classified as a priority pollutant and hazardous agent by international agencies ([4]). The World Health Organization ([5]) classifies pesticide safety according to the results obtained through LD_{50} tests on laboratory rats. Under this system, endosulfan is therefore

ranked by the WHO as Class II moderately hazardous. However, the United States Environmental Protection Agency (EPA) rates endosulfan as Category I b highly hazardous ([2]). The LD_{50} data for endosulfan are equivocal with some published results indicating that the chemical should be in the WHO's Class I b, according to the organizer's own criteria. Evidences of the threats to human health posed by endosulfan are abundant and the chemical has been banned or severely restricted in over 30 countries ([6]). Safe application, however, cannot be guaranteed under conditions of use in the developing countries where the chemical is still widely used.

In Kashmir valley, the use of endosulfan has been increased on apple orchards due to an outbreak of the red-mite in some years. This has resulted in bird kills and also been linked to the decline of fish catch (population), size and weight in the Dal Lake ([1]). Endosulfan runoff from agricultural fields killed fish in masses in various parts of the world ([2]). Also, research has shown that exposure to endosulfan, even at sublethal doses, induces behavioral and biochemical changes in fish. It has been found that larval (Salamander) activity and growth rate declined following endosulfan exposure ([7]). Oxidative stress and declines in cell viability have recently been observed in endosulfan-exposed rainbow trout ([8]). Increased lipid peroxidation in liver, kidney and gill of the freshwater fish Channa punctatus was found after 24 hr of low level endosulfan exposure ([9]). Under sub-lethal conditions, endosulfan depressed oxygen consumption and red blood cell count in the freshwater fish Clarius dussumieri ([10]). Recently, alterations in movement behavior were found associated with increased brain lipid peroxidation in the freshwater fish mirror carp exposed to lead ([11]). The present study evaluates Endosulfan-exposed changes in hematological events, behavioral activity and brain lipid peroxidation in mirror carp. We have also tested the efficacy of antioxidant action of selenium against endosulfan-induced behavioral and peroxidative stress.

METHODS

CHEMICALS, ANIMALS AND HEMATOLOGY

Chemicals and reagents used in this study were of analytical or highly purified grade. Endosulfan technical (Thiodan 35% EC, by Bayer Cropscience India Limited, Mumbai) was purchased from a local market. 2-thiobarbituric acid was purchased from Sigma Chemical Company (St Louis, MO, USA). Living fish specimens of mirror carp, Cyprinus carpiovar specularis (14 ± 2 cm in length, weighing 70 ± 10 g) were taken from local freshwater sources in the present study. Laboratory conditions were maintained as described elsewhere ([11],[12]). Briefly, the light and dark cycle of 12 hr was maintained throughout the study. Water characteristics were temperature 22 ± 2 °C, pH 7.8 ± 0.4, alkalinity 95 ± 5 ppm and dissolved oxygen 9 ± 1 ppm. These parameters were measured according to the procedures given in Adoni 1985 ([13]). Fish were accommodated in adequately large enough tanks. They were quarantined and acclimatized for a week prior to the experiment. They were randomly assigned to control (0 hr exposure or unexposed) and experimental (exposed for 120 and/or 240 hr) groups, each having 6 animals. Fish were exposed to endosulfan (1 microg/L) and/or endosulfan and sodium selenite (1 microg/L and 5 microg/L, respectively) for 120 and 240 hr. Movement behavior test was performed on individual fish in an aquarium. All the experiments were performed separately. Hematological parameters were studied by routine methods ([14]) in control (0 hr or unexposed) and endosulfan exposed (120 and 240 hr) groups.

OPEN-FIELD BEHAVIOR ACTIVITY

Motor activity study was performed in an aquarium (60 × 30 × 30 cm, L × W × H). The floor of the aquarium was equally divided with lines of 2 cm of intervals crossing each other squarely. The side wall was divided into three horizontal lines. The movement of fish by one segment as marked on the floor was recorded as one count. Crossing of one horizontal line upward or downward by fish was recorded as two counts. Whereas, one count was awarded for one upward or downward movement of fish by its half-length.

TISSUE PREPARATION

The fish were decapitated and their brains were carefully removed and placed in a Petri dish on an ice bath. After carefully removing the meninges together with surface blood vessels, the brain was weighed separately. Samples were prepared in triplicate. Brain was homogenized in chilled 0.15 M KCl using a Teflon pestle to give a 5% w/v homogenate.

LIPID PEROXIDATION ASSAY

The quantitative assay of malondialdehyde (MDA), as an index of lipid peroxidation, was performed by the 2-thiobarbituric acid (TBA) reaction method ([15]) as follows. One milliliter of brain homogenate was aerobically incubated at 37 °C in a water bath-cum-metabolic shaker (180 strokes /min of 2 cm amplitude) for 2 hr. The solution was immediately cooled with tap water and the formation of

lipid peroxidation was stopped by the addition of 1 mL cold 10% w/v trichloroacetic acid (TCA). For zero-time reaction, brain homogenate was taken with 1 mL of 10% w/v TCA in another test tube. The solution was thoroughly mixed and centrifuged at 2000 rpm for 10 min. In another test tube, 1 mL of supernatant was taken and allowed to react with an equal volume of 0.67 % w/v TBA for 10 min in a boiling water bath. The mixture was cooled with tap water and diluted with 1 mL of double distilled water. The absorbance was recorded at 535 nm in a spectrophotometer. The results were expressed as nmol of MDA formed per 30 min (the molecular extinction coefficient of MDA, E_{535}= 1.56 ×10^5; ($_{16}$).

STATISTICAL ANALYSIS

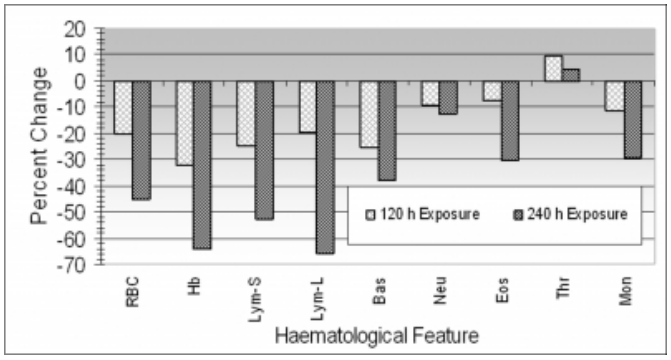
Statistical analysis was performed using SPSS (11.5 version), SPSS Inc, USA. Data were subjected to Paired-Samples T Test. Significant differences were considered if the p-values were less than 0.05 or otherwise mentioned. Lipid peroxidation findings were accessed in a Box–Whisker Plot for a clear presentation of the data to understand at a glance the statistical features.

RESULTS

Endosulfan, at low sub-lethal dose, altered all the blood features in fish (Figure 1).

Figure 2

Figure 1: Hematological features of fish exposed to endosulfan (1 microg/L, for a period of 240 hr). Values indicate percent change versus the control.



The red blood cell (RBC) population was observed to be declined by 20% (P<0.05) at 120 hr and by 45.2% (P<0.01) at 240 hr of endosulfan exposure. The hemoglobin (Hb) levels were also depressed by 32.2 % (P<0.05) at 120 hr and 64.3 % (P<0.01) at 240 hr exposure. The decrease of 24.6 % (P<0.05) of counts of small lymphocytes (Lym-S) was noted at 120 hr which further declined (52.6%, P<0.01) at 240 hr.

The counts of large lymphocytes (Lym-L) were found to be lowered in endosulfan-affected fish by 19.5 at 120 hr (P<0.05), and 65.9% (P<0.01) at 240 hr. Suppression of the basophil (Bas) numbers was observed at 120 hr (25.0%, P<0.01) and by 37.9% (P<0.01) at 240 hr of endosulfan treatment. Attenuation of neutrophils was 9.4% (NS) at 120 hr and 12.5% (P<0.05) at 240 hr of the pesticide exposure. The eosinophils (Eos) were diminished by 30.4% (P<0.01) at 240 hr. The thrombocyte (Thr) was the only blood parameter studied showing elevated counts (9.5%, P<0.05) at 120 hr. The monocytes (Mon) were depressed by 11.7% (P<0.05) at 120 hr and by 29.5% (P<0.01) at 240 hr in endosulfan-exposed fish.

Endosulfan developed and/or altered many behavioral responses in fish during the span of exposure. The major behavioral events shown were jerky behavior, violent movements, excited or hyper activity, short movements, turbulent activity, respiratory distress or whip-like activity during the period of endosulfan exposure. The violent behavior appeared first then followed by other behaviors. However, maximum or multiple behavioral responses were apparent during 4 and 6 days of exposure to endosulfan. The respiratory distress and whip-like response dominated in the last two days of exposure.

The movement behavior, reported as the distance moved, was significantly depressed at 240 hr (P< 0.05) compared with corresponding control and endosulfan-exposed (120 hr) groups. Synchronized treatment of Se with endosulfan significantly enhanced the response of movement behavior at 120 hr (P< 0.05) and 240 hr (P< 0.01) compared with the corresponding endosulfan exposed groups (Table 1).

Figure 3

Table 1: Effects of endosulfan (1 microg/L) and endosulfan and sodium selenite (1 microg/L and 5 microg/L, respectively) on movement behavior of fish.

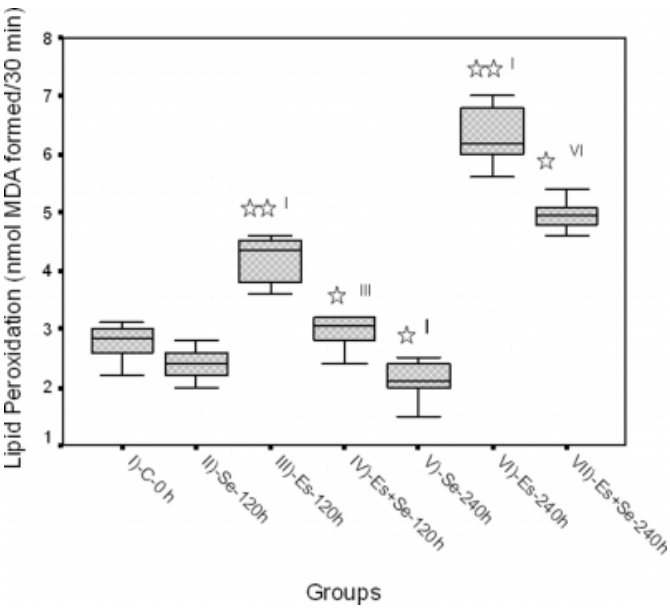
Group	Exposure Period	
	120 hr	240 hr
Control	90.00 ± 1.44	84.00 ± 1.65
Endosulfan	66.00 ± 1.18 (– 26.70)[a]	18.00 ± 0.86[*a, c] (– 78.57)[a]
Selenium	114.00 ± 1.41[*a] (26.00)[a]	115.00 ± 1.29[*a] (36.00)[a]
Endosulfan + Selenium	76.00 ± 1.88[*b] (15.15)[b]	35.00 ± 0.86[**b] (94.44)[b]

Values expressed as movement score, Mean ± SEM. Figures in parentheses indicate % change. a = compared with corresponding Control group, b = compared with corresponding Endosulfan group, c = compared with Endosulfan (120 hr) group. * = $P < 0.05$, ** = $P < 0.01$, Paired-Samples T Test.

Lipid peroxidation values simultaneously increased as the treatment time of endosulfan progressed (51.6%, at 120 hr and 127.4% at 240 hr, both P<0.001). It showed maximum variability at both the exposure periods. When endosulfan-exposed fish were concurrently treated with Se, lipid peroxidation in the brain was decreased (P<0.002) compared to endosulfan-exposed group at 120 hr. Further at 240 hr, lipid peroxidation was also suppressed by Se (P<0.002) compared with the corresponding endosulfan-exposed group. Moreover, these two groups exhibited the least variability as shown by inter-quartile deviation range (Figure 2).

Figure 4

Figure 2: Effects of endosulfan (1 microg/L) and endosulfan and sodium selenite (1 microg/L and 5 microg/L, respectively) on lipid peroxidation (expressed as nmol of malondialdehyde formed per 30 minutes) in fish brain. Significant values represent * at P<0.05, and ** at P<0.001 as compared to control (I) or corresponding control (III, VI) groups.



Nevertheless, after termination of endosulfan exposure on 240 hr, lipid peroxidation returned to almost normal values at or after the period of 480 hr when Se treatment was subsequently prolonged (for further 240 hr, data not shown).

DISCUSSION

In the present investigation, it has been revealed that low level sublethal exposure of endosulfan affects hematology, behavioral and locomotion activity, and brain lipid peroxidation in fish. Also, supplementation of Se relieved the toxic manifestations of endosulfan in fish on their movement behavior and brain lipid peroxidation. Almost all the measured blood parameters in fish were found affected by endosulfan exposure at both test periods. The longer exposure period had greater toxic response over the shorter one. Moreover, the trend of anomalies in the blood events differed at 120 240 hr exposure. The rank order of the percent decline of the values at 120 hr was: Hb > Bas > Lym-S > RBC > Lym-L > Mon > Neu > Eos; Thr showed an elevated response. At 240 hr exposure on the other hand, the rank order of the percent decrement was greater and followed: Lym-L > Hb > Lym-S > RBC > Bas > Eos > Mon > Neu. Furthermore, the decreasing rank order was analogous for the Lym-S and the RBC at both of the

exposure periods. In an earlier report, endosulfan was found to depress the RBC counts at 48 hr exposure ([10]).

Endosulfan exposure caused behavioral development and/or alterations in fish seen during the study period. In early periods of exposure, jerky and violent behaviors developed in fish, followed by short movements and excitations. Subsequently, a comparatively longer span in respiratory deficiency or discomfort was shown in fish (during 120 hr and 240 hr). However, whip-like behavior in fish dominated in the last two days. Symptoms of sublethal poisoning of endosulfan were also observed in amphibians that included hyperactivity, whip-like convulsions, temporary paralysis and slow growth rate ([17]). In our experiment, the movement behaviors of fish (observed in an open field test) showed spontaneous declines at both test periods. It appears from the present study that endosulfan induced some toxic behavioral manifestations and depressions in locomotor activity in fish. To see whether the endosulfan-induced depression of locomotion could be reversed, we tested selenium. It is well established now that Se is an essential micronutrient. Observations have also suggested that Se influences compounds with hormonal and neurotransmitter activity in brain, and that could be the reason that Se affects moods and behavior in animals ([18]). Our observations clearly indicate that Se treatment alleviated the behavioral anomaly of hypo-motor activity (seen as decreased movement) in fish caused by endosulfan exposure.

Studies have shown that endosulfan toxicity resulted in increased lipid peroxidation in liver, kidney and gills of a fish ([9]). Earlier, investigation from this laboratory has shown elevated lipid peroxidation in brain and altered behavior in lead-exposed fish ([11]). In this study, endosulfan-exposed fish were examined for lipid peroxidation in brain. It has been found that endosulfan enhanced lipid peroxidation in fish brain for both test periods. A recent study demonstrated protective effect of Se against lipid peroxidation damage ([19]). Unlike many metals, Se is a required micronutrient for fish. Also, Se is a component of glutathione peroxidase (GPX) and presumably protects against lipid peroxidation induced cellular damage by destroying toxic peroxides ([20]). On the other hand, Se deficiency results in reduced GPX activity and Se content in the brain ([18]). In this sense, Se was tested for its protective role against brain lipid peroxidation. Thus, Se attenuated endosulfan-induced accentuations of lipid peroxidation in fish brain at both test periods of 120 hr and 240 hr. The results suggest that Se may well play a beneficial role in reducing the toxic oxidative damage in the brain and relieving fish from deteriorating movement behavior caused by endosulfan.

ACKNOWLEDGEMENT

I am highly thankful to Engineer Mubashir R. Qadri, of the SKUAST-K Workshop for help in fabrication of the aquarium. My thanks are to Dr. A. A. Khan, Senior Asstt. Professor, Division of Statistics & Economics for help in statistical analysis and, Professor M. A. Balkhi, Head Division of Fisheries, for encouragements. I specially thank to Mr. Bashir Ahmad Shah, Chief Technician, Central Laboratory, SMHS Hospital, Government Medical College, Srinagar, for his valuable technical help in hematological analysis.

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