Effect of organochlorine pesticide endosulfan on blood plasma of fresh water teleost, *Anabas testudineus*

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SUMMARY

Endosulfan is an organochlorine pesticide which is less persistent in the environment but more toxic to fish than other organochlorines. An acute static renewal test was conducted to determine the LC_{50} concentrations of endosulfan for 24, 48, 72 and 96h. For stress response study, the healthy male fishes were exposed to 7 ppb (LC_{10} 96 hour) of endosulfan for 48 hours and sampling were made at different time intervals of 0, (control) 2, 12, 14, 20, 24, 36 and 46 hours and recovery for a period of 48 hour (sampled at 2,12 and 48 hours) were studied after transferring the 48 hour exposed animal to toxicant free water. Several new bands which were not present in the control plasma were detected at different time intervals (20, 24, 36, and 46 hour) in the treated plasma and these bands had tendency to disappear during the recovery phase (2, 12 and 48 hour) and were comparable to the known protein bands in the molecular weight markers. Since these bands appeared during exposure period and disappeared in recovery phase, it seemed to be stress induced.

Key words : Endosulfan, LC50, Stress response, *Anabas testudineus*

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The environmental stress and physiological adaptations go hand in hand and the success of the organisms mostly depends upon how best it can adapt and cope up with the changed environmental conditions. There are many definitions of stress in fish. According to Brett (1958) "the stress is a state produced by an environmental or other factor which extends the adaptive responses of an animal beyond the normal range or which disturbs the normal functioning to such an extent that in either case, the chances of survival are significantly reduced". However, it is seen that when the physiological and/or psychological range and patterns are extended beyond their normal range, the organisms are subjected to a state of stress. Normally pollutants are the common environmental stressors, which can produce alterations in the normal physiology of the organisms. According to Hans Selye (1973) stress is "the nonspecific response of the body to any demand made upon it". Quite simply, stress can be considered as a state of threatened homeostasis that is re-established by a complex suite of adaptive responses (Chrousos, 1998). The stress, depending on its magnitude and duration, may affect fish at all levels of organization, from molecular and biochemical to population and community (Adams, 1990). Hence, the present attempt was under taken to study the stress response induced by endosulfan on plasma proteins of *Anabas testudineus*.

MATERIALS AND METHODS

Live specimen of adult fresh water fish, *Anabas testudineus* were collected locally. The fish weighing 45–50 g with the length of 14-15 cm were brought to the laboratory and acclimatised to the laboratory conditions for 15 days in large glass aquaria. All experimental glass aquaria were cleaned and filled with 40 L of seasoned (chlorine-free) tap water prior to the experiment. The test solution was prepared from the commercial endosulfan (35 EC). An acute static renewal test was conducted to determine the 24, 48, 72 and 96h LC_{50} concentration of endosulfan. Three replicates each containing eight fish were exposed to each concentration (3, 5, 7, 9, 14, 16, 18, 20, 25, 28, 31, 35, 38 and 40 ppb) of endosulfan. A blank solution (00 ppb) in three replicates each of eight fish was used as control. The media (control and test solution) in the aquaria were renewed daily (Bindu and Geetha, 2008).

For stress response study, the healthy male fishes of body weight 40-50g were selected from the holding tanks and segregate to three batches and simultaneously exposed to 7 ppb (LC_{10} 96 hour) of endosulfan. The experiment was run for the period of 48 hours and sampling were made at different time interval of 0, (control) 2, 12, 14, 20, 24, 36 and 46 hour and recovery for a period of 48 hour (sampled at

2,12 and 48 hours) were studied after transferring the 48 hour exposed animal to toxicant free water. Batches of animals (N = 3) exposed to endosulfan were sampled from experimental system at specific intervals. Immediately after sampling, blood was collected from the ventral aorta to heparinised vaccutainers and was transferred into separately labeled eppendrofs and stored in refrigerator. The samples were then centrifuged in an eppendrof centrifuge (eppendrof centrifuge 5810 R) for 10 minutes at 10000 rpm. The plasma collected was diluted four times with sample buffer (62.5 mM Tris-HCl, P^H 6.8. 1% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.1% bromophenol blue) and boiled at 100° C for 3 – 5 minutes and immediately used for electrophoresis.

Plasma samples were analysed using a 15 well gel. Each well was loaded with 20 µl plasma. Electrophoresis was performed under denaturing and discontinuous conditions on 12% sodium dodecyl sulfate (SDS) polyacrylamide gel (PAGE) using a vertical gel unit as per Laemmli (1970) and Titus (1991). The molecular weight markers were also run along with the samples. The plasma samples loaded in the wells were electrophoresed at a constant current of 60V for stacking and 120V for separating gel at 16°C in an electrophoresis buffer (TGE pH-8.3). Electrophoresis was performed until the bromophenol blue dye front has run near the bottom of the gel.

After the running of the gel, the gel was separated and stained in 0.25% coomassie brilliant blue R 250 in a mélange of 50% methanol and 10% acetic acid overnight and destained in a solution of 10% methanol and acetic acid to get the best quality protein bands. The stained gels were fixed in 7.5% acetic acid at 4°C. The appropriate molecular weights of resolved proteins were determined by comparison with known standards.

Statistical analysis:

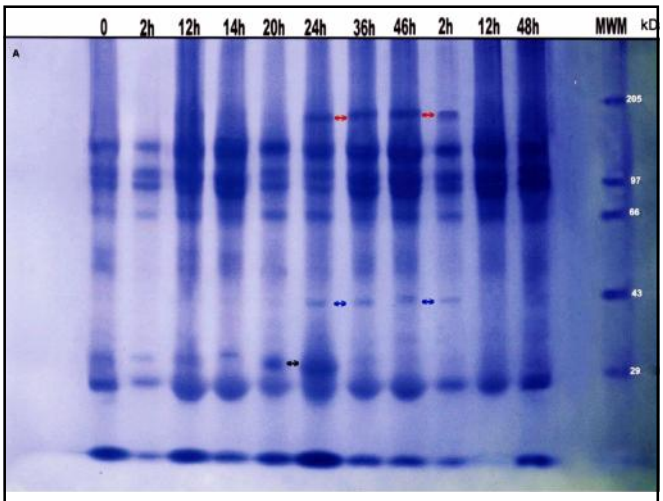
LC_{50} values of endosulfan at different exposure periods *i.e.* 24, 48, 72 and 96 hour were determined, by following a computerized statistical package namely SPSS 14.0. Concentration at which 10% deaths occured at 96 hour was taken as lethal concentration. The safe concentration was taken as $1/100^{th}$ value of 96 hour LC_{50} as suggested by Indian Standard Institution.

RESULTS AND DISCUSSION

LC_{50} values of endosulfan (35 EC) for *A.testudineus* at different exposure periods were determined. Median lethal concentrations for 24, 48, 72 and 96h were 27.58, 23.35, 18.79 and 16.91 ppb, respectively. The test result of the LC_{50} of the present study at 96h was 16.91 ppb, which indicated that endosulfan is very highly toxic to fish. Several workers have estimated median lethal concentration (LC_{50}) of endosulfan for fish. It has been suggested that LC_{50} of endosulfan for most of the fishes may vary from 0.0006 to 10 ppm (Gupta and Gupta, 1979; Gupta *et al*., 1984).

Comparison of different bands appeared in the electropherogram revealed variations in the plasma proteins following exposure to lethal concentration (7ppb) of endosulfan. Several new bands which were not present in the control plasma were detected at different time intervals (20, 24, 36, and 46 hour) in the treated plasma and these bands tended to disappear during the recovery phase (2,12 and 48 hour) and were comparable to the known protein bands in the molecular weight markers. The most apparent one was that detected between 97 kDa and 205 kDa in treated plasma. This band was seen in the plasma sampled at 24, 36 and 46 hours and it was most concentrated at 46^{th} hour. In the recovery phase of 2, 12 and 48 hour, this protein band showed gradual disappearance. In addition to this, another protein band having approximate molecular weight of 30 kDa was detected at 20 and 24 hours and it wass most concentrated at 24^{th} hour. This protein band was not seen in 36 and 46 hour. During the recovery phase, this protein band disappeared. Another protein band having approximated molecular weights of 43 kDa appeared in the treated plasma sampled at 24, 36 and 46 hours. In the recovery phase of 2, 12 and 48 hour, this protein band tended to disappear (Fig. 1)

Fishes display a wide variation in their physiological responses to stress. The response to stress is considered an adaptive mechanism that allows the fish to cope with real or perceived stressors in order to maintain its normal or homeostatic state. Quite simply, stress can be considered as a state of threatened homeostasis that is re-established by a complex suite of adaptive responses (Chrousos, 1998). If the intensity of the stressor is overly severe or longlasting, however, physiological response mechanisms may be compromised and can become detrimental to the fish's health and well-being, or maladaptive, a state associated with the term "distress" (Hans Selye, 1974; Barton and Iwama, 1991). In the present study, the electropherogram clearly revealed the appearance of some new plasma proteins following endosulfan treatment in the male fresh water fish, *Anabas testudineus*. The intensified protein band between 97 kDa and 205 kDa appeared in endosulfan treated plasma was presumed to be vitellogenin. The vitellogenin identified in the plasma of fish had molecular weight usually ranging from 100-200 kDa (Allner *et al*., 1999; Hiramatsu *et al*., 2002). Vitellogenin having the



Band 1, Red makr : plasma protein that appeared between 97 k Da and 205 kDa
Band 2, Blue mark : plasma protein with approximate molecular wieght of 43 kDa.
Band 3, Black mark : plasma protein with approximate molecular weight of 30 kDa

Fig. 1 : Electropherogram of plasma of *Anabas testudineus* exposed to endosulfan for 48 hours and sampled at different time interval of 9 (control), 2, 12, 14, 20, 24, 36 and 46 hours and recovery for a period of 48 hours, sampled at 2, 12 and 48 h.

molecular weights of 145 and 150 kDa was detected in the plasma of cat fish, *Ameiurus nebulosus* (Roach and Davies, 1980) and surface water fish, *L. indicus* (Allner *et al*., 1999). Endosulfan treated plasma in the present study, also revealed the presence of 30 and 43 kDa protein bands. Appearance of protein bands having the molecular weight of 30 and 180 kDa in the plasma of *Oreochromis mossambicus* treated with cortisol, corticosterone, DES, progesterone and testosterone was reported by Sunny *et al*. (2002).

The presence of vitellogenin is considered as a biomarker for the detection of estrogen and estrogenic compounds in the aquatic medium (Funkenstein *et al*., 2000). A wide range of environmental contaminants possessing estrogenic activity have been reported and may be associated with domestic sewage (Soto *et al*., 1995; Guillette *et al*., 1996). More over endosulfan is a synthetic chlorinated cyclodiene that is an environmental endocrine disruptor (Soto *et al*., 1995). Vitellogenin is normally synthesized in the liver of female oviparous vertebrates. It is estrogen dependent and increases markedly in the serum during oocyte development (Wallace, 1985; Specker and Sullivan, 1994).The environmental compounds affect the biochemical messenger system of fish by acting as agonists or antagonists (Colborn *et al*., 1993). Endocrine disrupting chemicals can alter endocrine function by a variety of mechanisms (Sonnenschein and Soto, 1998) : Mimic the effect of endogenous hormones, Antagonize the effect of endogenous hormones, Disrupt the synthesis and metabolism of endogenous hormones, Disrupt the synthesis and metabolism of hormone receptors.

In male fishes vitellogenin is not synthesised under normal condition. But the presence of estrogen or estrogenic compounds in the aquatic medium stimulates the synthesis of vitellogenin in males. The present study is just a priliminary attempt to assess the stress response and endocrine disruption of endosulfan (35EC), to aquatic organisms especially fishes. More detailed studies are needed in this field to establish the role of endosulfan as an endocrine disruptor.

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