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Dose and Duration Dependent Impact of Tributyltin (TBT) on ATP ases of Chick Brain

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ABSTRACT

TBT is antifouling agent also categorized under persistent organic pollutant that affects many organs. The present study was designed to assess the dose and duration dependent in vivo toxic effects of TBT on membranebound ATPases like Total, Na+ K+, Ca++, Mg++, Ca++ HCO3-, Mg++ HCO3- ATPases of brain tissue of male chick. Two separate animal groups were exposed by intramuscular administration of 60 and 600 µg kg-1 bw day-1 doses of TBT for continuous 6, 12 and 18 days of exposure durations. Parallel untreated group was also maintained. The results indicated that TBT evoked disturbances in the specific activity of estimated ATPases in various degrees. Although the overall results indicated that different TBT doses did not produce any significant toxic effects on ATPase activity, at the same time significant exposure duration dependent effect was profound in ATPase activity of chick brain.

KEYWORDS : TBT Toxicity, Male chick, ATPases, Brain

INTRODUCTION

The toxicity of Tributyltin compounds has become unique focusing point for research because of the extensive uses of TBT includes biocide in paints and coatings used for marine antifouling applications, preservative for wood, textiles, paper, leather (White et al. 1999). In the 1980s, concerns about the evidence toxicity of TBT to non target species led to restricted use of TBT or uses under government regulations among many industrialized countries. Despite such restrictions, TBT persists in many areas at levels considered to be chronically toxic to the most susceptible organisms (Stab et al. 1995; Cardwell et al. 1999). The toxic effects of TBT compounds has been reported in different test species, organ and different cells of organism and its mode of action was explained in multiple ways (Fent 1996; EFSA 2004; Inadera 2006). It seems to be capable to interrupt cellular components and physiological processes. Food chain accumulation of tributyltin (TBT) has been shown in meat and fish products (Kannan et al. 1996, 1998; Iwata et al. 1997; Hoch 2001).

Due to characteristic of high lipophilicity of TBT (Rudel 2003), biological membranes have been considered first targets for its mode of action (Gadd 2000). Stridh et al. (1999) reported that low concentrations of TBTC triggered an immediate depletion of intercellular ATP followed by necrotic death in Jurket cells and showed that the mode of cell death was typically apoptotic. Heywood and Waterfield (1989) recorded changes of membrane structure, such as lysis, caused by tributyltin compounds and suggested that this could lead to an increased permeability.

MATERIALS AND METHODS

Chemicals and Experimental animal

Bis-tributyltin oxide, ($C_{24}H_{54}OSn_2$), with purity 96% was used to prepare TBT doses by dissolving it in corn oil. Chick (White leghorn strain, "Broiler") male represented as experimental animal. The experiment was started with two days old animals.

Experimental design

Animals were divided into three groups. In which Control group was received only corn oil as it was used as vehicle for the toxicant. Animals of second group were received 60 µg kg^{-1}bw TBT dose $day^{-1}$and named as toxicated 1 group. Animals of remaining group were received 600 µg kg^{-1} bw TBT dose day^{-1} and named as toxicated 2 group. Route of exposure for TBT doses was intramuscular. To accomplish three exposure durations viz., 6, 12 and 18 days, animal of each group was sacrificed on 7th, 13th and 19th day of experiment respectively.

Collection of ATPase enzyme fraction

On the scheduled days brain tissue was dissected and homogenized into chilled sucrose-EDTA-Imidiazole buffer (pH 7) then centrifuged at 7,000 RPM. Thus formed pellet was resuspended in a sucrose-EDTA-Imidiazole-Deoxycolate disodium salt buffer (pH 7) and further centrifuged at the same speed to obtain ATPase enzyme fraction. All steps were carried out at 0-4°C.

Assay of ATPases enzyme activity

Activities of Total, Na^+K^+, Ca^{++}, Mg^{++}, Ca^{++} HCO_3^- and Mg^{++} HCO_3^-ATPases were estimated as per the method of Zaugg (1982) with appropriate modifications by Lakshmi et al. (1991). In the sample, concentration of inorganic phosphate (P*i*) hydrolyzed by known amounts of protein, which is an indirect measure of ATPase activity, was spectrophotometrically evaluated according to Fiske and Subbarow (1925) method. Specific activity of ATPase was expressed as µmoles P*i* mg $protein^{-1}$ h^{-1}. The protein content present in the sample was determined as per the method of Lowry et al. (1951).

Statistical analysis

Variability in generated data between employed sub lethal doses of TBT and among three different exposure durations were calculated by Two Way ANOVA test. t test was also calculated between control and individual toxicated group.

RESULTS

Results clearly indicated that TBT changes the usual activity of estimated membrane ATPases of chick brain. Due to given different sub lethal doses of TBT, the activity of Total ATPase showed stimulation in all exhibited exposure durations except after 18 days, animals treated with 600 µg kg^{-1} tried to reach up with the enzymatic activity of control group (Fig. 1a). The activity of Na^+K^+ ATPase was noted similar as observed in Total ATPase only exception was observed after 12 days of exposure duration, where the enzymatic activity was inhibited by 600 µg kg^{-1} dose of TBT (Fig. 1b).

Within initial two exposure durations (6 and 12 days), Ca^{++} ATPase was suppressed by given lower dose (60 µg kg^{-1}) as well as higher dose (600 µg kg^{-1}) of TBT. On the other hand in the last duration (18 days), stimulation was observed in both toxicated groups (Fig. 1c). The activity of Mg^{++} ATPase was inhibited by lower dose (60 µg kg^{-1}) of TBT after initial two sequential exposure durations (6 and 12 days). Moreover, after 18 days the activity was stimulated as compared to enzymatic activity of control. Whereas, because of 60 µg kg^{-1} dose of TBT after 6 days the activity was stimulated and in mid duration (12 days) try to reach up to the activity of control and in last duration (18 days) once again stimulation was observed (Fig. 1d).

The activity of $Ca^{++}HCO_3^-$ ATPase showed similar effects as reported in Mg^{++} ATPase except after 18 days of exposure duration the enzymatic activity of $Ca^{++}HCO_3^-$ ATPase was inhibited by given lower dose (60 µg kg^{-1}) of TBT (Fig 1e). The activity of $Mg^{++}HCO_3^-$ ATPase was stimulated by given both sub lethal doses (60 and 600 µg kg^{-1}) of TBT as compared to their respective control, during 6 and 18 days of exposure duration. However, the activity was inhibited and stimulated due to

60 and 600 µg kg^{-1} dose, respectively in 12 days of duration (Fig. 1f).

DISCUSSION

The present study examines whether the different doses or different exposure durations causes toxic effect of TBT on brain ATPases. Two way ANOVA clearly suggests that TBT intoxication by different sub lethal doses does not have significant effect on estimated membrane bound ATPase enzyme system. However, different exposure durations causes significant changes on enzyme system (Table 1).

Table 2 shows 't' test between control and individual toxicated group. The activity of Total ATPase showed significant variation only in case of lower dose group (60 µg kg^{-1}) after initial two exposure (6 and 12) durations. When t test was calculated between control and toxicated 1 group, it was noted that the activity of Na^+ K^+ ATPase showed significant changes only after 6 days of exposure duration. When t test was calculated between control and toxicated 1 group, the activity of Ca^{++} ATPase showed significant variations in all three exhibited exposure durations. In addition, toxicated 2 group showed significant changes after initial exposure duration. The activity of Mg^{++} ATPase showed variation between control and toxicated 2 group after 6 and 18 days of exposure durations. In addition, t test between control and toxicated 1 group showed significant changes after 12 days of exposure. Results of t test between control and toxicated 1 suggested that $Ca^{++}HCO_3^-$ ATPase showed changes only after 12 and 18 days of exposure duration. In case of $Mg^{++}HCO_3^-$ ATPase, the activity of this enzyme showed significant changes between only control and toxicated 1 group after 18 days of exposure duration.

CONCLUSION

In brain tissue, alteration in ATPase enzyme activity was due to different exposure durations of TBT. It might be possible that different sub lethal doses of TBT did not produce that much toxic effect on brain ATPase system. So, the results of the study indicative of time dependent variation in the selected ATPase enzyme activity in brain tissue.

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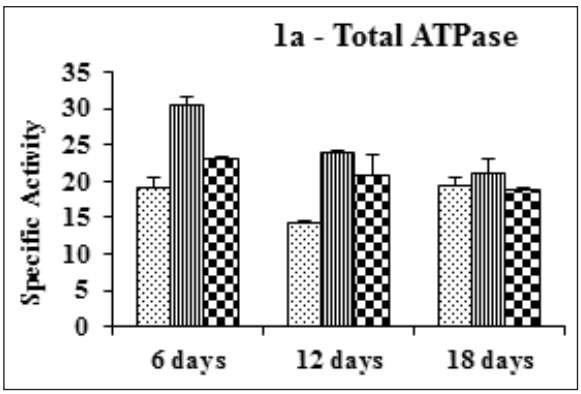
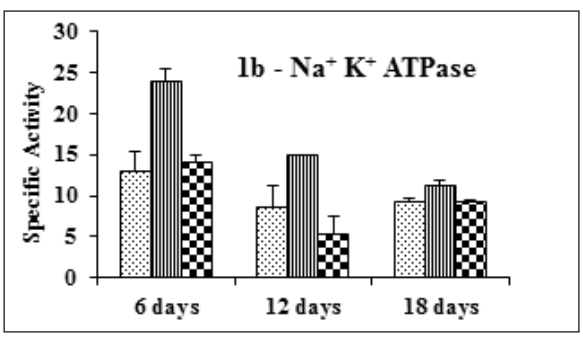
Table 1. Two Way ANOVA of different enzymes. The given F critical value for between doses is **5.318** and for among durations is **3.438**. '*' denotes statistical significance at P = 0.05 level.

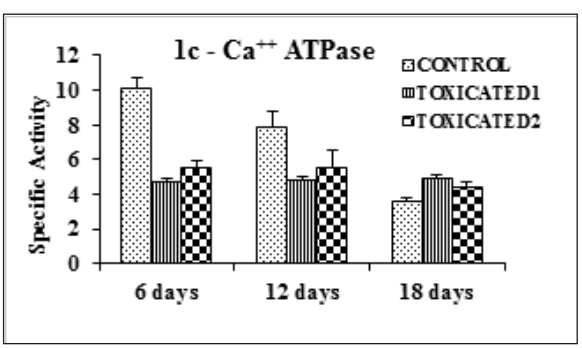
Enzymes	Between doses	Among durations
Total ATPase	0.767	18.509*
Na^+ K^+ ATPase	1.086	22.470*
Ca^{++} ATPase	0.001	22.915*
Mg^{++} ATPase	0.062	13.676*
$Ca^{++}HCO_3^-$ ATPase	0.007	18.424*
$Mg^{++}HCO_3^-$ ATPase	4.037	3.456*

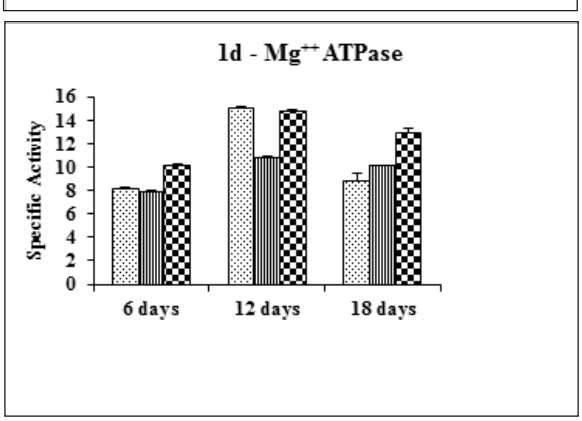
Table 2. Student's 't' test between control and individual toxicated group. The given critical value of 't'=**4.303** and '*' denotes statistical significance at P = 0.05 level.

Enzymes	6 DAYS		12 DAYS		18 DAYS	
	C Vs T1	C Vs T2	C Vs T1	C Vs T2	C Vs T1	C Vs T2
Total ATPase	8.842*	3.942	28.091*	3.105	0.960	0.539
Na^+ K^+ ATPase	5.188*	0.582	3.430	1.347	2.942	0.024
Ca^{++} ATPase	11.267*	8.302*	4.453*	2.350	7.689*	2.775
Mg^{++} ATPase	2.381	11.324*	25.727*	2.338	2.697	6.719*
$Ca^{++}HCO_3^-$ ATPase	2.383	1.656	7.158*	1.644	9.230*	2.207
$Mg^{++}HCO_3^-$ ATPase	1.016	1.942	1.050	0.260	8.519*	3.364









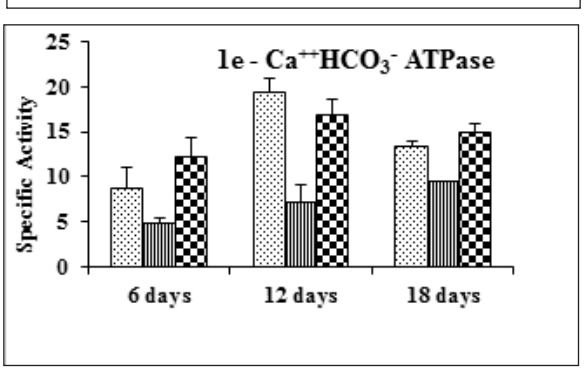
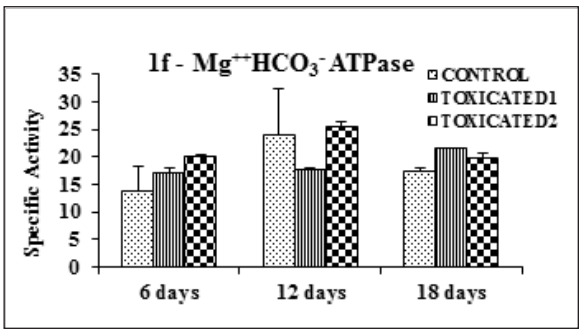


Figure 1. Alteration in the specific activity of ATPases in brain tissue of developing chick. Data represented by mean of specific activity ± SD.



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