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# BIOCHEMICAL TARGETS AFFECTED BY SUB-LETHAL DOSES OF LINDANE AND DELTAMETHRIN

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## ABSTRACT

The effect of daily oral administration of sub-lethal doses of lindane (5 mg/kg) and deltamethrin (2 mg/kg) for three weeks on male mice after one hour and after three weeks of last dose were studied. The treated mice showed reduction of body weight. There was also a significant increase in liver, kidney and brain:body weight ratios after three weeks of last dose. In contrast, there was a significant decrease in spleen:body weight ratio.

The tested pesticides significantly inhibited mice brain ATPases (Total-ATPase, $Na^+$, $K^+$-ATPase and $Mg^{2+}$-ATPase) after one hour and after three weeks of treatment except that deltamethrin insignificantly inhibited $Mg^{2+}$-ATPase. Pesticides failed to produce any significant changes in the levels of $\gamma$-amino butyric acid (GABA) and glutamic acid.

## INTRODUCTION

Insecticidal pyrethroids and organochlorine compounds are well known to be directly toxic to nerves (Narahashi, 1985). The nervous system is controlled by many neurotransmitters and their receptor proteins. Glutamate and its decarboxylated derivative $\gamma$-aminobutyric acid (GABA) are neurotransmitters, that mediate excitatory and inhibitory transmission, respectively, in the central nervous system (CNS) of vertebrates and invertebrates (Cotman and Iversen, 1987 and Robinson and Coyle, 1987).

Many authors found that pyrethroids and organochlorines inhibited the activities of $Na^+$, $K^+$ and $Mg^{2+}$-ATPase in both insects and higher animals (Bakry, et al., 1984 and Morshedy, 1989). This suggests a possible interference in energy metabolism required for ion transport across nerve membranes (Murphy, 1980).

The present study is devoted to investigate the effect of lindane (organochlorine) and deltamethrin (pyrethroid) on some biochemical parameters in mice in an attempt to clarify some of the mechanisms underlying the toxicological side effects of these agents. The general physiological state of the mice was monitored by the change in body weight during the experiments. Also, the liver, kidney, heart, spleen and brain:body weight ratios of treated mice were looked at and compared with the controls to show the effects of the tested pesticides on each organ.

## MATERIALS AND METHODS

Animals: Male Swiss albino mice strain (Mus musculus), weighing 25-30 gm were used in this study. They were obtained from the High Institute of Public Health, Alexandria University.

Pesticides: Lindane, 99%, introduced by Shell Chemical Co., New York, U.S.A. and Deltamethrin, 98%, introduced by Sumitomo Chemical Co.

### Experimental Groups

The male mice were divided into three groups. The animals were orally administered for three weeks with 5 mg lindane/kg(first group); 2 mg deltamethrin/kg (second group) and corn oil (third group) as control. The administered doses represent 1/10th of the $LD_{50}$ of each pesticide (Worthing and Walker, 1983). The weight of the animals were recorded daily. Five mice from each group were sacrificed after one hour and after

three weeks of last dose. The liver, kidney, heart, spleen and brain:body weight ratios of treated mice were measured and compared with the controls. The brains were immediately frozen at -20°C until used.

## Biochemical Studies

### Assay of the activity of brain ATPases

The whole brain was homogenized in 10 volumes of 40 mM tris-HCl buffer containing 0.32 M sucrose and 1 mM EDTA, pH 7.4. The homogenate was centrifuged at 5000 xg for 20 minutes. The supernatant was centrifuged again at 12,000 xg for 30 minutes. Then the pellets were resuspended in the same buffer. The enzyme activity was measured as reported by Koch (1969). The total ATPase activity was determined in a reaction mixture containing 100 mM-$Na^+$, 20 mM-$K^+$, 5 mM-$Mg^{2+}$, 5 mM-ATP, 40 mM tris-HCl buffer pH 7.4 and 0.1 ml of the enzyme. The reaction was stopped by adding 150 µl of tricholoroacetic acid followed by determination of inorganic phosphate (Pi) using the method described by Taussky and Shorr (1953). $Mg^{2+}$-ATPase activity was measured after addition of 1 mM ouabain to the above reaction mixture before the addition of ATP. $Na^+$, $K^+$-ATPase activity was obtained by subtraction of $Mg^{2+}$-ATPase activity from the total ATPase activity. Protein content was assessed using the method of Lowry, et al. (1951). The specific activity was expressed in µmole inorganic phosphate released/ milligram protein/ hour.

### Determination of brain GABA and L-glutamate content

The chromatographic method used was a modification of the procedure described by Maynert, et al. (1962) and Pepeu, et al. (1970). The whole brain was homogenized in seven volumes of ethanol solution. The precipitated protein was removed by centrifugation at 3000 xg for

15 minutes. The clear supernatant fluid was evaporated to dryness, the residue was dissolved in distilled water. An amount of clear supernatant fluid was applied to a Whatman filter paper No. 1. The chromatogram was run by ascending technique using a mixture of n-butanol, acetic acid and water as a solvent. Then the dried strips were dipped in 0.5% (w/v) ninhydrine. After the color developed, each spot was separately cut out and eluted in ethanol solution. The optical density of the eluate was measured at wavelength of 570 nm. The amount of each amino acid present in each spot was calculated using the standard curve.

Statistical analysis is done using Student's t test to compare between treated mice and control.

## RESULTS AND DISCUSSION

### I. Effect of repeated oral administration of sub-lethal doses of lindane and deltamethrin on the body weight and organ weight

Table 1 presents the effect of sub-lethal doses of lindane and deltamethrin (5 & 2 mg/kg, respectively) given for a period of three weeks on body weight of male mice. The data revealed significant decrease in body weight during the three weeks of treatment, reached the maximum at the last day of treatment. After stopping the treatment the body weight began to increase, reached maximum in lindane treatment at the end of experiment. But, the percentage mean of body weight gain was still less than the control group. It is clear that the rate of increase of body weight after stopping the treatments was faster in lindane treatments than in deltamethrin ones.

Our results are in agreement with the results of Vos and Krajnc (1983) and El-Masry (1987) who found that pesticides caused a marked loss of weights as compared with control animals.

**Table 1**

Effect of repeated oral administration of sub-lethal doses of lindane and deltamethrin on the rate of body weight change of male mice.

| Time interval | % mean** of gain or loss of body weight±SD | | |
|---|---|---|---|
| | Control | Deltamethrin | Lindane |
| Within treatment | | | |
| 1st week | +13.23 ±2.99 | -4.79 ±2.80* | +2.07 ±1.07 |
| 2nd week | +20.29 ±3.85 | -7.68 ±5.56* | -11.11 ±8.77* |
| 3rd week | +29.22 ±2.53 | -8.8 ±5.78* | -11.26 ±8.54* |
| After treatment | | | |
| 1st hour | +32.47 ±5.04 | -6.58 ±7.08* | -8.27 ±5.17* |
| 1st week | +38.32 ±5.14 | -3.3 ±1.02* | +1.5 ±3.00* |
| 2nd week | +40.42 ±6.50 | -1.73 ±0.09* | +10.59 ±2.96* |
| 3rd week | +45.92 ±4.64 | -0.75 ±0.04* | +13.37 ±2.29* |

(+): means gain of body weight.
(-): means loss of body weight.
*: significantly different from control ($P<0.05$).
**: mean was taken from five mice and three replicates of each.

In contrast, these results disagree with that reported by Davies and Holub (1978) who mentioned that no significant differences in the final body weight between parathion-treated animals and the corresponding control were observed.

Table 2 illustrates the effect of lindane and deltamethrin on liver, kidney, heart, spleen and brain: body weight ratios for male mice after 1 hour and after 3 weeks of last treatments. From this Table, it was obvious that the treated mice showed increase in liver, kidney and brain:body weight ratios. However, the increase was not significant after 1 hour of the two insecticides, but was significant after 3 weeks. Our results are in agreement with Nigam, et al. (1982). There was no change in the ratio of heart to body weight in treated and untreated mice. This indicates less toxic effect of the two used pesticides on that organ. On the other hand, there was a significant decrease in spleen:body weight ratios, these results were supported by Berberian (1981), who found that the spleen weight decreased significantly in rats injected with DDT pesticide. It is possible that the changes in organ weight are a part of a generalized increase in cellular metabolism.

## II. Effect of repeated administration of lindane and deltamethrin on different targets of male mice brain.

### A. ATPases

Table 3 shows the mean values of the specific activities of ATPases expressed as μ mole Pi/mg protein/hr. It is clear that brain was much higher in $Na^+$, $K^+$-ATPase (4.28 ± 0.82) than $Mg^{2+}$-ATPase (1.26 ±0.09) by about three fold. This result indicated that $Na^+$, $K^+$-ATPase constituted most of the enzymatic ATPase activity of the mice brain. This data was relatively similar to that found by Morshedy (1989).

The effect of sub-lethal doses of tested

Table 2

Effect of repeated oral administration of sub-lethal doses of lindane and deltamethrin on the liver, kidney, heart, spleen and brain: body weight ratio after 1 hour and 3 weeks of last treatment.

| | % Mean** ±SD (Organ: Body weight ratio) | | | | |
|---|---|---|---|---|---|
| | | Lindane | | Deltamethrin | |
| | Control | 1 hr | 3 weeks | 1 hour | 3 weeks |
| Liver | 4.95 ±0.70 | 5.10 ±2.00 | 6.48 ±0.77* | 5.00 ±0.28 | 6.50 ±0.45* |
| Kidney | 0.73 ±0.10 | 0.84 ±0.89 | 0.92 ±0.03* | 0.78 ±0.07 | 0.98 ±0.014* |
| Heart | 0.56 ±0.12 | 0.54 ±0.08 | 0.55 ±0.04 | 0.53 ±0.05 | 0.54 ±0.01 |
| Spleen | 0.43 ±0.17 | 0.39 ±0.04 | 0.30 ±0.09* | 0.38 ±0.13 | 0.21 ±0.08* |
| Brain | 1.30 ±0.42 | 1.41 ±0.32 | 1.62 ±0.15* | 1.53 ±0.17 | 1.61 ±0.18* |

*: Significantly different from control value ($P<0.05$).
**: Mean was taken from five mice and three replicates of each.



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Table 3

Effect of repeated oral administration of sub-lethal doses of lindane and deltamethrin on different targets of male mice brain after 1 hour and 3 weeks of last treatment.

| Treatment | Mean ±SD<br>Activity of ATPases µmole/mg protein/hr<br>Total-ATPase | <br>$Na^+K^+$-ATPase | <br>$Mg^{2+}$-ATPase | GABA conc. µg/gm brain | Glutamate conc. µg/gm brain |
|---|---|---|---|---|---|
| Control | 5.46 ±0.98 | 4.28 ±0.82 | 1.26 ±0.09 | 225.53 ±10.08 | 756.22 ±15.56 |
| Lindane | | | | | |
| 1 hour | 4.22 ±0.85* | 3.25 ±0.61* | 1.03 ±0.02* | 230.86 ±9.56 | 776.50 ±11.23 |
| 3 weeks | 3.82 ±0.28* | 2.68 ±0.14* | 0.98 ±0.11* | 236.66 ±8.65 | 724.28 ±12.36 |
| Deltamethrin | | | | | |
| 1 hour | 4.28 ±0.36* | 3.30 ±0.18* | 1.21 ±0.10 | 260.18 ±16.08 | 825.32 ±7.86 |
| 3 weeks | 4.06 ±0.32* | 3.18 ±0.20* | 1.12 ±0.09 | 271.28 ±10.32 | 859.31 ±6.36 |

*: Significantly different from control value ($P<0.05$).
**: Mean was taken from five mice and three replicates of each.

pesticides on the activity of ATPases was illustrated in Table 3. Lindane and deltamethrin significantly inhibited the activity of mice brain ATPases after one hour and after three weeks of treatment, except that deltamethrin insignificantly inhibited $Mg^{2+}$-ATPase. Lindane was more potent than deltamethrin, and the percentage inhibition is higher after three weeks than after one hour of treatments in all ATPases. Lindane and deltamethrin inhibited total ATPase by 30.40% and 25.64%, respectively, after 3 weeks of treatment. El-Demerdash (1987) found that the percentage inhibition of total ATPases reached 33.7 after repeated administration of lindane in mouse brain. As shown in Table 3 deltamethrin did not significantly change the $Mg^{2+}$-ATPase in mouse brain. The percentage inhibition reached 4.76 and 11.11 after one hour and after three weeks of treatment, respectively. This result is in agreement with Bakry, et al. (1984). who concluded that the inhibition of mitochondrial $Mg^{2+}$-ATPase is not the primary mode of insecticidal action of pyrethroids, while Clark and Matsumura (1982) stated that cypermethrin and decamethrin mainly inhibited Ca, Mg-ATPase. Morshedy (1989) found that the decamethrin and permethrin had no effect on rat brain $Na^+$, $K^+$-ATPase, while dicofol and DDT exerted more effect.

The results generated from the present study confirmed that lindane and deltamethrin exerted more effect on mice brain $Na^+$, $K^+$-ATPase. This may be explained as being due to the fact that $Na^+$, $K^+$-ATPase is a lipoprotein and that it requires phospholipids for optimal activity (Morero, et al., 1972). It is therefore possible that lindane and deltamethrin and/or their lipophilic metabolites may bind to the lipid moiety in vivo which may result in altering the allosteric characteristics of the enzyme, thus leading to the inhibition of its activity.

## B. GABA and Glutamate

The levels of GABA and glutamate in mouse brain expressed in µg/gm brain tissue were 225.5 and 756.2, respectively. In the present study, daily subcutaneous of either lindane (5 mg/kg) or deltamethrin (2 mg/kg) for 21 days failed to produce any significant changes in the levels of GABA and glutamate of the male mice brain after both one hour and 21 days of treatments. This indicated that the mechanism of action of tested pesticides does not involve these neurotransmitters.

These results are in accord with the findings of Lock and Berry (1981) and Hong, et al. (1984), who found that kepone did not affect the concentration of glutamate and GABA in different regions of rat brain. They also, agree with El-Demerdash (1987) and Saad (1990), who found that some organo-chlorine compounds and synthetic pyrethroids did not significantly alter the concentration of glutamic acid and GABA in rat or mice brain.

The results generated from the present study showed that, due to the high lipid solubility of lindane and deltamethrin, the mechanism of their actions on the ATPase complex can be due to a direct interaction with the lipid portion of ATPase leading to a disturbance of lipid-protein interaction which is important for the activity of ATPase. Also the data does not suggest that GABA and glutamate as neurotransmitters in the mammalian CNS, are sensitive sites for the toxic actions of lindane and deltamethrin.

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# تأثير الجرعات تحت المميته من مبيدى اللندان والدلتامثرين على بعض الاهداف البيوكيميائيـــــه

ويهدف هذا البحث دراسة تأثير مبيدى اللندان والدلتامثرين بالجرعات تحت المميته ( ٥ مجم / كجم ، ٢ مجم / كجم على التوالى ) على بعض الثوابت البيوكيميائيـــــه لفئران الالبينو . وقد تم رصد التغير فى وزن جسم الحيوان اثناء التجربه والنسبه بـــين وزن الاعضاء المختلفه ( الكبد ـ الكليه ـ المخ ـ القلب ـ الطحال ) الى وزن الجســـم الكلى فى الحيوانات المعامله ومقارنتها بالكنترول لمعرفة تأثير المبيد على كل عضو .

وقد أظهرت النتائج انخفاض فى وزن الجسم للافراد المعامله عن الكنتـــــرول بالاضافه الى حدوث زيادة واضحه فى النسبه بين وزن الكبد والكليه والمخ الى وزن الجسم بعد ثلاث اسابيع من آخر معامله ، وعلى العكس ظهر انخفاض معنوى فى النسبه بـــين وزن الطحال الى وزن الجســـم .

ويتضح من النتائج ايضا ان المبيدان المستخدمان لم يحدثا تغيير محسوس فـــى مستوى كل من حامض جاما أمينوبيوتريك ، وحامض الجلوتاميك . بينما حدث تثبيـــط واضح فى نشاط انزيمات الادينوسين ثلاثى الفوسفاتيز ( الكلى ـ الصوديوم والبوتاسيـــوم ـ الماغنسيوم ) بعد ثلاث ساعات وثلاث اسابيع من آخر معامله ما عدا مبيد الدلتامتريـــن فكان تثبيطه غير واضح على أنزيم الماغنسيوم ادينوسين ثلاثى فوسفاتيز .