EFFECTS OF DIFFERENT DOSE OF CYPERMTHRIN ON SERUM ACETYLCHOLINE CONCENTRATION, SPINAL CORD AND SCIATIC NERVE HISTOPATHOLOGY IN ADULT RATS.

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(Received18September 2013,Accepted31 Octeber 2013)

Keywords: cypermethrin .serum acetylecholine , rats.

ABSTRACT

The present study aimed to investigate the effects of different doses of of Cypermthrin on serum acetylcholine concentration , Spinal cord and Sciatic nerve histopathology in adult rats. Fourty eight adult rats were used , they divided randomly and equally into four groups . Control group orally dosed with normal saline for 90 days . The other three groups were dosed orally with different three doses of cypermthrin , high dose (64 mg / kg b.w.) intermediate dose (32 mg / kg b.w.) and low dose (16mg / kg b.w.). The results shows that serum acetylecholine concentrations increased significantly ($p \leq 0.05$) in rats exposed to high and intermediate dose of cypermethrine compared with control group. Whereas there were no significant difference ($p \geq 0.05$) between low dose of cypermethrine and contol group . Hitopathological examination of spinal cord and Sciatic nerve revealed that there were a dose dependent increase in vaccuolation in nerves fibers to be affect larg number of nerve fiber in high dose and it affect few numbers of nerve fibers in low dose. In conclusion cypermthrin affected positively histopathological findinds of nerves fibers .

INTRODUCTION

Cypermthrin is a synthetic pyrethroid which is applid topically for the control of ectoparasites such as ticks , fleas, lice and blowflies. It consists of a mixture of 4cis-and 4trans-isomers. The ratio of cis- trans –isomers in commercial products depend on the manufacturing source. (1)

Over ninety percent of the cypermethrin manufactured worldwide is used to kill insects on cotton. It is also used on lettuce and pecans, to kill cockroaches (and other indoor pests) in buildings, and to kill termites (2).

Cypermethrin, like all synthetic pyrethroids, kills insects by disrupting normal functioning of the nervous system. In insects, as well as all other animals including humans, nerve impulses travel along nerves when the nerves become momentarily permeable to sodium atoms, allowing sodium to flow into the nerve. Pyrethroids delay the closing of the "gate" that allows the sodium flow (3). This results in multiple nerve impulses instead of the usual single one. In turn, these impulses cause the nerve to release the neurotransmitter acetylcholine and stimulate other nerves (4).

Cypermethrin has been identified as one of the important constituent pesticides associated with human health risks (5).

Alfa-cypermethrin (α−CP) (two of the four cis-isomers of cypermethrin), is the most potent cypermethrin and is being extensively used in agricultural farming, livestock industry and to control household ectoparasites to protect human health (6).

In spite of wide range of effectiveness, cypermethrin (CY) is not free from side effects. Signs like muscular tremors, ataxia, weakness of limbs, convulsions, coma and death from respiratory depression have been reported after ingesting high doses of CY, while its dermal contact in the facial area may cause a subjective sensation of tingling or numbness (7).

Depending on the fact that cyermethrin is a toxic material to lab animals the present study was designed to determine the neuro-histopathological effects of cypermethrin and serum acetylecholine concentrations in rats

MATERIALS AND METHODS

In this study 48 adult rats were used , they divided randomly and equally into four groups . Control group orally dosed with normal saline. The other three groups were dosed orally with different three doses of cypermthrin , high dose (64 mg / kg b.w.) intermediate dose (32 mg / kg b.w.) and low dose (16mg / kg b.w.) for 90 days.

1.Determination of acetylcholine (ACH)

The serum ACH was estimated by ELISA test for the quantitative determination of ACH concentration in rat serum by using (CUSABIO , China) ELISA kit.

- **Principles of sandwich ELISA**

The test procedure was done as in manual of (CUSABIO , China) ELISA kit.

2-Histopathological parameters

- **Procedure of Tissue Processing**

In brief the routine sequence of events according to (١٣ and ١٤) is as follows:-

After obtained the tissue. Fix it for 24 hours or more in an appropriate fixative buffered formalin 10%. Dehydrate through ascending alcohol (increasingly higher concentration) alcohols overnight. And then Replace alcohol (clear) with xylol or chloroform. Then infiltrate with paraffin. Embed in a block of paraffin. Cut thin sections on the microtome (5µm- thick). Mount the section on glass slides. And remove (dissolve) the embedding medium by putting the slides on hot plate overnight. Then rehydrate the sections in descending alcohols. Stain the section with an appropriate staining sequence (H&E).

Staining Procedure

In the staining procedures used haematoxylin and eosin stains according to (١٥) for paraffin sections.

Statistical Analysis :

The data were subjected to analysis of variance and the significance differences at ($p<0.05$) which were determined by (ANOVA), one-way by using the statistical softwares sigmastat statistical (Version 19.0,SPSS Inc., Chicago, Illinois, USA, 2010).

RESULTS

(A) Effects of cypermethrine on Serum acetylecholine concentration :

The results in table (1) revealed that serum acetylecholine concentrations increased significantly ($p \leq 0.05$) in rats exposed to high dose of cypermethrine (64mg/kg.bw) and intermediate dose of cypermethrine (32mg/kg.bw) compared with control group. There were on significant($p \geq 0.05$) difference between high dose of cypermethrine and intermediate dose of cypermethrine when compared with each other. Whereas there were no significant difference ($p \geq 0.05$) between low dose of cypermethrine (16mg/kg.bw) and contol group .

Table (1) Serum concentrations of acetylecholine in rats exposed to different doses of cypermethrine compared with control group.(mean ± SD)

Group	Acetylecholine Con.
Group 1 (high con.)	12.70 ± 01.15 A
Group 2 (intermediate con.)	10.26 ± 01.41 A
Group 3 (low con.)	08.56 ± 00.66 B
Group 4 (control)	07.59 ± 00.84 B

Different letters represent significant difference between groups at ($p \leq 0.05$).

(B) Effects of cypermethrine on spinal cord and sciatict nerve histopathology :

The histopathological results of spinal and sciatc nerve revealed presence of dose dependent vacuolation of nerve fibers **(figure 1, 2 , 3 , 4 , 5 , 6 , 7 , and 8) .**

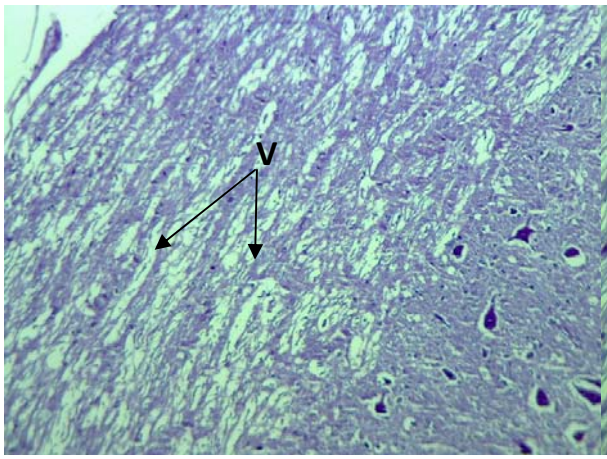

figure (1) Cross section in spinal cord of rats exposed to cypermethran (16 mg / kg b.w.) for 90 days . E.&H. x 100.(A) prominent vaccuolation(V) In white matter

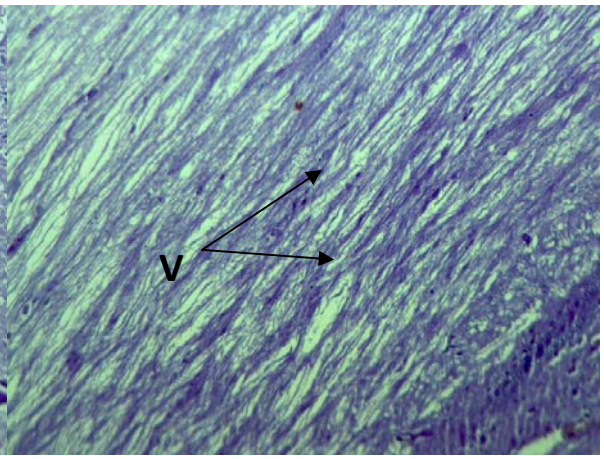

figure (2) Cross section in spinal cord of rats exposed to cypermethran (32 mg / kg b.w.) for 90 days . E.&H. x 100. larg numbers of nerve fibers affected by vacculation(V) in white matter .

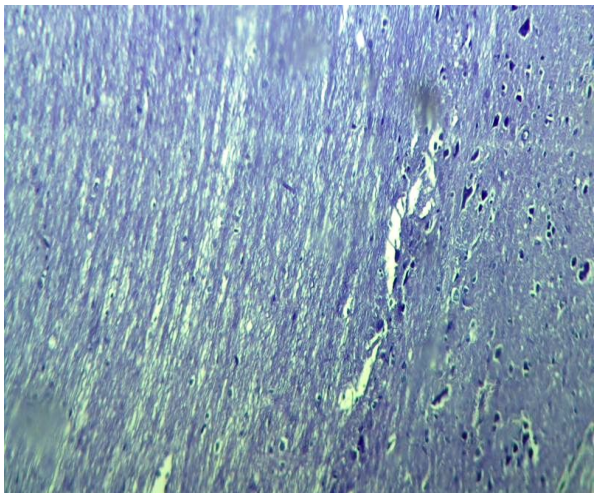

figure (3) Cross section in spinal cord of control group rats shows normal feature of spinal cord . E.&H. x 100.

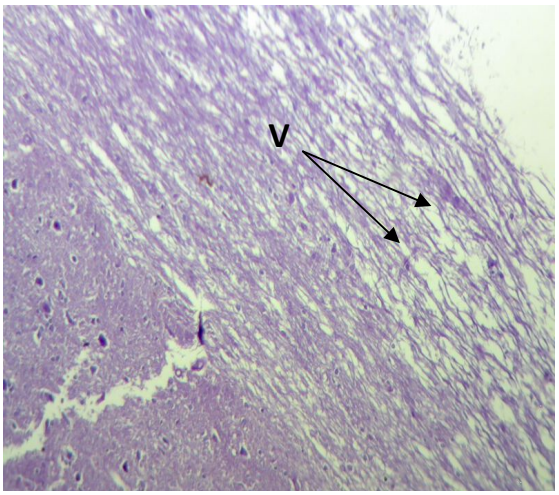

figure (4) Cross section in spinal cord of rats exposed to cypermethran (64 mg / kg b.w.) for 90 days .shows larg numerous nerve vacculation(V) in white matter of spinal cord . E.&H. x 100.

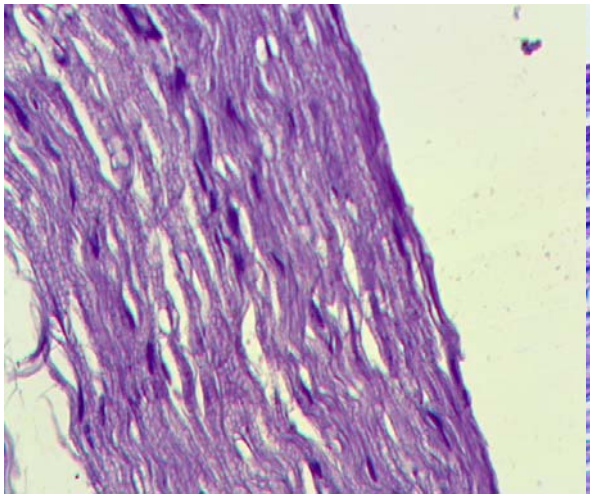

figure (5). Cross section in schiatic nerve of control rats shows normal structure . E.&H. x 400.

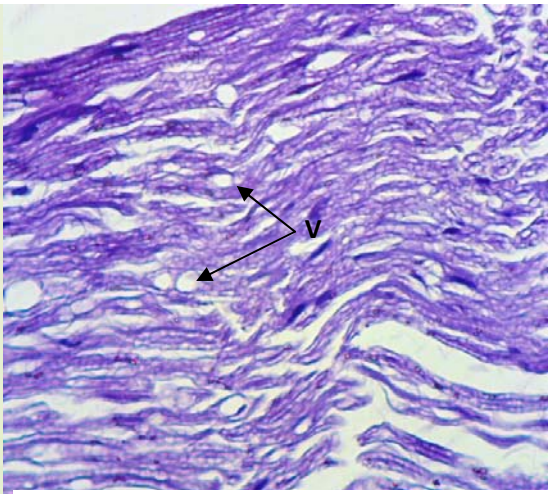

figure (6). Cross section in schiatic nerve of rats exposed to cypermethran (64 mg / kg b.w.) for 90 days . shows prominent vaccuolation(V) in white matter . E.&H. x 400.

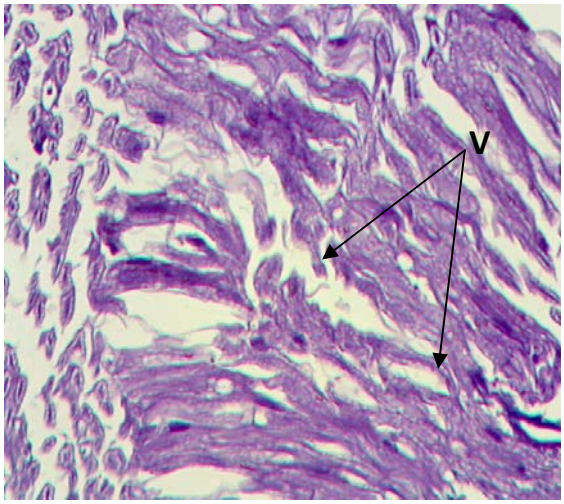

figure (7). Cross section in schiated nerve of rats exposed to cypermethran (32 mg / kg b.w.) for 90 days . shows few vaccuolation(V) in white matter . E.&H. x 400.

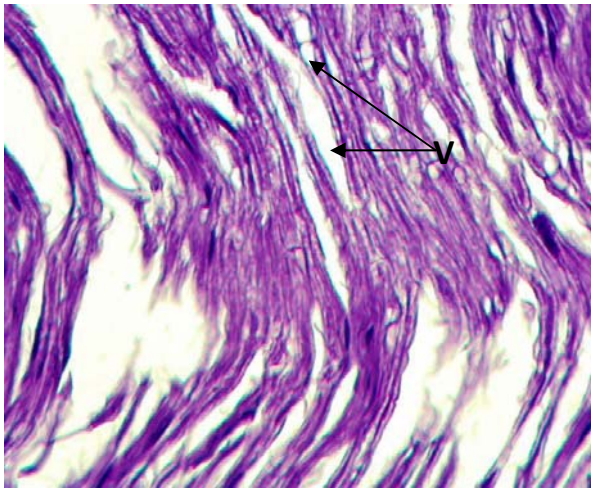

figure (8). Cross section in schiated nerve of rats exposed to cypermethran (16 mg / kg b.w.) for 90 days . shows few vaccuolation(V) in white matter . E.&H. x 400.

DISCUSSION

The results of the present study revealed that serum acetylecholine concentrations increased significantly in rats exposed to high and intermediate dose of cypermethrine compared with control group(table-1-). The possible cause that may explain this increase in serum acetylecholine is inhibition of acetylecholine estrease which consequently cause increase serum acetylecholine. The results of the present study came in agreement with (8) who reported that cholinesterase activities decreased in the cypermethrine treated animals. According to (9) Cholinesterase was markedly depressed to a different degree in plasma and brain of animals receiving cypermethrine.

The histopathological results of spinal and sciatc nerve revealed presence of dose dependent vacuolation of nerve fibers .According to (10) who noticed that a very rapid distribution in the nervous system within five minutes after intravenous administration in rats. While, (11) reported that a swelling myelin sheath and breaking of some axons of sciatic nerves as a result for cypermethrin effects on barky sheep . The results of the present study agreed with (12) who reported that cypermethrin generate vacuolation of the sciatic nerve in pigeons with low, intermediate and high dose of cypermethrin founded with degenerate vacuolated nerve fibers.

تأثير الجرع المختلفة من السايبرمثرين على تركيز استايل كولين المصل والتغيرات النسجية المرضية للحبل الشوكي والعصب والوركي في الجرذان البالغة

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الخلاصة

اجريت الدراسة الحالية لاختبار تأثير الجرع المختلفة من السايبرمثرين على تركيز الاستايل كولين في المصل ، التقطيع النسيجي المرضي للحبل الشوكي و العصب الوركي في الجرذان البالغة . استخدم في هذة الدراسة ثمانية واربعون من الجرذان البالغة ، قسمت عشوائيا وبصورة متساوية الى اربعة مجاميع . اعتبرت المجموعة الاولى كمجموعة سيطرة وجرعت المحلول الفسلجي لمدة ٩٠ يوما. اما المجاميع الثلاثة الباقي فقد جرعت فمويا بثلاث جرع مختلفة من السايبرمثرين ، جرعة عالية (٦٤ ملغم / كغم من وزن الجسم) ، جرعة متوسطة (٣٢ ملغم / كغم من وزن الجسم) وجرعة منخفضة (١٦ ملغم / كغم من وزن الجسم) . أظهرت النتائج وجود ارتفاع معنوي ($p \leq 0.05$) في تركيز الاستايل كولين في مصل الجرذان المعرضة

الى الجرعة العالية والجرعة المتوسطة من السايبرمثرين مقارنة مع مجموعة السيطرة ، في حين لم تكن هناك فروقات معنوية بين الجرعة المنخفضة ومجموعة السيطرة .أظهر الفحص النسيجي للحبل الشوكي و العصب الوركي ان هناك زيادة في تفجي الليف العصبي مع زيادة الجرعة المستخدمة ، ليكون هناك اعداد كبيرة من الالياف العصبية المتأثرة عند الجرع العالية والمتوسطة في حين ان هناك اعداد قليلة من الالياف العصبية المتأثرة عند الجرعة المنخفضة. يستنتج من الدراسة الحالية ان للسايبرمثرين تأثير سلبي على التقطيع النسيجي المرضي للاعصاب.

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