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(RESEARCH ARTICLE)

Effect of alpha-cypermethrin on serotonin, nor-epinephrine and brain changes in rats

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Abstract

In the present study, brain damages, serotonin and nor-epinephrine activity on rat brain were investigated. A single sub-acute lethal -oral dose of Alpha-Cypermethrin (25 mg kg $^{-1}$) was given to rat. Blood and brain tissue samples were examined biologically and histopathologically at various times (1, 2, 3, 4 and24 h) after dosing. Results revealed that extracellular serotonin levels % (f mol/mg tissue) in rat ventral hippocampus at 25 mg kg^{-1} B.W. After 1, 2, 3 and 24 hr. were 105±4, 80 ±7, 80 ±4, 70 ±4, respectively. While extracellular NA levels after 1, 2, 3 and 24 hr. were 300±10, 450 ±14, 400±12, 400±11, respectively. Alpha-cypermethrin acts as nor-epinephrine reuptake inhibitors (NRIs) and serotonin reuptake stimulator in rat brain. The lesions in the brain of the treated groups were gliosis, perineuronal vacuolation, perivascular vacuolation, and neuronal degeneration.

Keywords: Brain Damage; Alpha-Cypermethrin; Acute Sub-Lethal Dose; Serotonin; Nor-epinephrine.

1. Introduction

Cypermethrin, an alpha-cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3 (2,2-dichlorovinyl) cyclopropane carboxylic acid, is one of the most commonly used pyrethroid II pesticides. It is an artificial compound pyrethroid, broad spectrum, biodegradable insecticide, and fast-acting neurotoxin with good contact and stomach action. It is used to control many pest infestations. In cases of accidental or deliberate ingestion of relatively large amounts of solutions containing pyrethroids, signs of neurotoxicity such as headache, muscle crumbs, convulsions, and coma have been reported [1,2].

The synthetic pyrethroids delay closure of the sodium channel, resulting in a sodium tail current that is characterized by a slow influx of sodium during the end of depolarization. Apparently the pyrethroid molecule holds the activation gate in the open position. Pyrethroids with an alpha-cyano group (e.g., fenvalerate) produce more prolonged sodium tail currents than do other pyrethroids (e.g., permethrin, bioresmethrin). The former group of pyrethroids causes more cutaneous sensations than the latter. /Synthetic pyrethroids/ [3].

Synthetic pyrethroids delay the closure of the sodium channel, resulting in a sodium tail stream characterized by a slow sodium flow during the end of depolarization. The pyrethroid molecule appears to stop an activation gate in open mode. Pyrethroids with the alpha cayano group (for example, fenvalerate) produce more sodium tail streams for longer periods than other pyrethroids (such as permethrin and bioremethrin). The previous group of pyrethroids cause more skin sensations than the latter. / Synthetic pyrethroids [3].

Pyrethroids Type II can inhibit the specific binding at or near the picrotoxin site of GABAA receptors in the brain of mic [4,5], and in particular inhibit the flow of GABA-based chloride [6].

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Synthetic pyrethroids are used to protect animals, crops, and humans from a wide range of pests [7]. In recent years, the application of compounds has increased several -fold due to their lower mammalian toxicity and limited soil constancy compared to organochlorine insecticides [8].

The purpose of present study was to extend our knowledge of the action of cypermethrin on brain tissue in an animal model. The aging human population is chronically exposed to cypermethrin through food consumption (pesticide residues) in food, drinking water from dug wells, deep wells and water mains in rural areas. Therefore, study of an extremely interesting issue of practical significance.

2. Material and methods

2.1. Experimental animals

48 males of the Wistar rats were used, weighing between 180 and 200 g. According the standard protocol, all rats were housed in a quiet, non-stressful environment for one week prior to the study. Rats were divided into two main groups (alpha -cypermethrin treated group and control group)

Animals were housed in the Animals House Unit in Faculty of Veterinary Medicine, Cairo University. They were kept in plastic cages with stainless steel wire lids; (bedded with wood shavings); on a standard laboratory feed diet. Animals were housed at constant room temperature (20-22 °C), 60% humidity and light cycle of 12h. /day. All rats were given 45–50 kcal/day normal rat chows during the experimental period. Animal care as well as the experimental procedures was in compliance with guidelines of ethical standards released by Cairo university policy on Animal Care and Use. In order to minimize animal's suffering, we intended only to use the adequate minimal number of animals requested to produce reliable scientific data. All efforts were made to minimize the number of animals and their suffering in this study through following the guidelines released by Cairo university policy on Animal Care and Use.

The Alpha-Cypermethrin treated group, (n = 20) were received Alpha-Cypermethrin orally by gavages at a dose of 25 mg kg^{-1}.

Control group, (n = 20).

2.2. Sampling

Blood samples were drawn by cardiac puncture at constant intervals; 1 hr., 2 hr., 3 hr. and 24 hr. respectively (four of each time) from the two main groups.

Brain samples were taken at constant intervals; 1 hr., 2 hr., 3 hr. and 24 hr. respectively, (four of each time) from the two main groups.

2.3. Determination of Alpha-Cypermethrin concentration in Blood plasma

Cypermethrin concentration was determined on an Agilent 7890A gas chromatography (GC) equipped with an electron capture detector (ECD) and a HP-5 capillary column (30 m x 0.32 mm x 0.25 µm). The injector was open at 280 °C with an injection volume of 1µl. the oven temperature was programmed to ramp from 100 to 275°C at a rate of 15°C min^{1}.

Recoveries of cypermethrin at different fortification levels, i.e., 0.05, 0.1, 0. 5 and 1 µg mL^{1}, were determined in five replicates to validate and evaluate the accuracy of the method. The recoveries obtained were in the acceptable range 80.8-88.4%. The coefficient variation of the methods (CV %) for repeatability ranged from 5.1 % to 13.9 %, within acceptable range. The limit of detection (LOD) was estimated to be 0.001 µg mL^{-1} of the tested pesticide, based on signal-to-noise ratio 3:1, and the limit of quantification (LOQ) of cypermethrin was 0.005 µg mL^{-1}, which yields a signal-to-noise ratio of 10.

2.4. Determination of the concentrations of 5-HT(Serotonin) and NA (Noradrenaline) in brain regions

Analytics: Concentrations of 5-HT and NA were determined within the same samples by HPLC separation and electrochemical detection [9]. Each sample was divided into two subsamples; one was used for 5-HT and other for NA analysis. Aliquots were injected onto the high-performance liquid chromatography (HPLC) column (Gilson, model 832).

2.5. Histopathological Investigation

Brain samples were excised, wiped with brine and then stabilized in 10% buffered formalin PH 7.0 (phosphate sprayer). In short, fixed specimens were dried and dehydrated, cleared in xylene and embedded in paraffin wax. The blocks were made, and 4 µm-thick sections were cut using a sledge microtome. Paraffin has been removed from sections of brain tissue and moistened and stained with hematoxylin and eosin (H&E). Stained slides were examined using a bright field light microscope to investigate the histological alteration in the structure of the brain.

2.6. Statistical analysis

SAS. (2000). (Statistical Analysis System, Version 9.2): SAS Institute Inc., Cary, NC 27513-2414 USA [10].

3. Results and discussion

Table 1 Concentrations of alpha-cypermethrin oral sub-lethal dose in blood plasma of rats given sub-lethal oral doses (25 mg kg-1 BW).

Time	Alpha-Cypermethrin
1hr	0.416±0.15
2hr	0.233±0.058
3hr	0.162±0.058
24hr	0.257±0.158

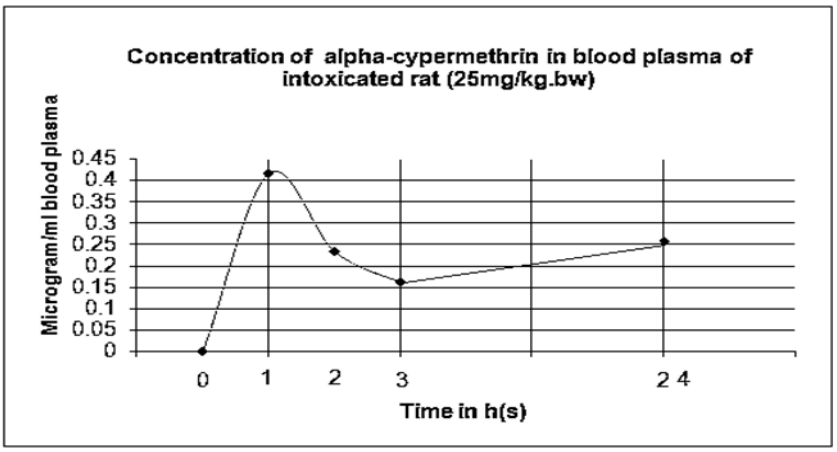

Figure 1 Concentrations of alpha-cypermethrin oral sub-lethal dose in blood plasma of rats given sub-lethal oral doses (25 mg kg-1 BW).

Table 2 Effects of alpha-cypermethrin oral sub-lethal dose on the extracellular serotonin and noradrenalin levels in rat ventral hippocampus.

Time	Alpha-cypermethrin oral sub-lethal dose		Control	
	¥Noradrenalin	¥Serotonin	Noradrenalin	Serotonin
1hr.	300±10	105±4	302±23	60.8±33
2hr.	450 ±14	80 ±7	350 ±12	58 ±7
3hr	400±12	80 ±4	300±12	60 ±45
24 hr.	400±11	70 ±4	350±33	59.3 ±42

¥: Basal level % (f mol/mg tissue: femoto mol).

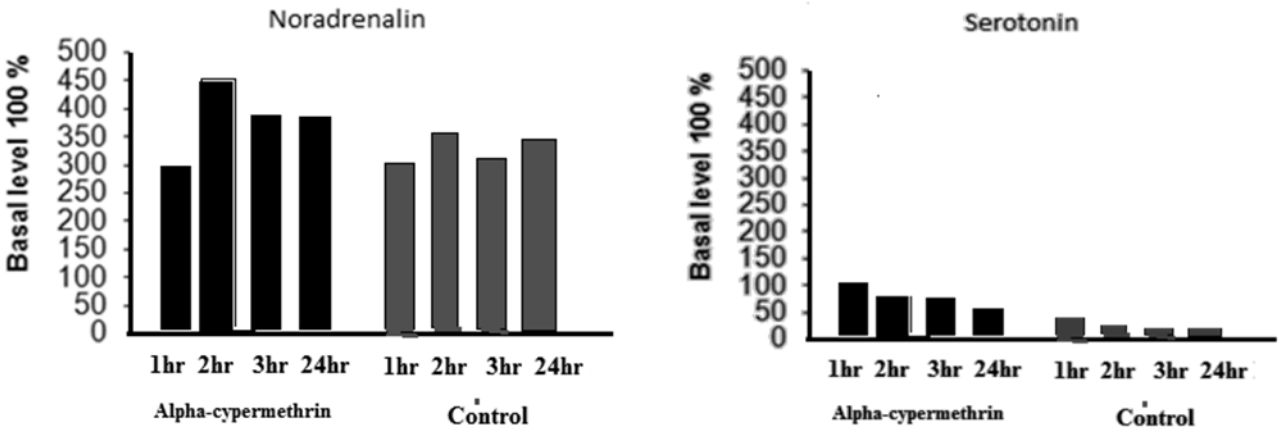

Figure 2 Effects of alpha-cypermethrin oral sub-lethal dose on the extracellular serotonin and noradrenalin levels in rat ventral hippocampus.

Table 3 Effects of Alpha-cypermethrin on the binding of [^{3}H] RX 821002 to α_2-adrenergic receptors in the rat brain (2 hours post treatment).

Brain region	Control	Alpha-cypermethrin (mg kg-1 B.W.)
Hippocampus formation	61.40±2.63	65.2±3.42*a
Cortex	128.46±3.24	138.25±4.77a
Thalamus	131.72±3.55	134.55±5.22b

*(F mol/mg tissue); a: Significant at P<0.5; b: Non-significant at P<0.5

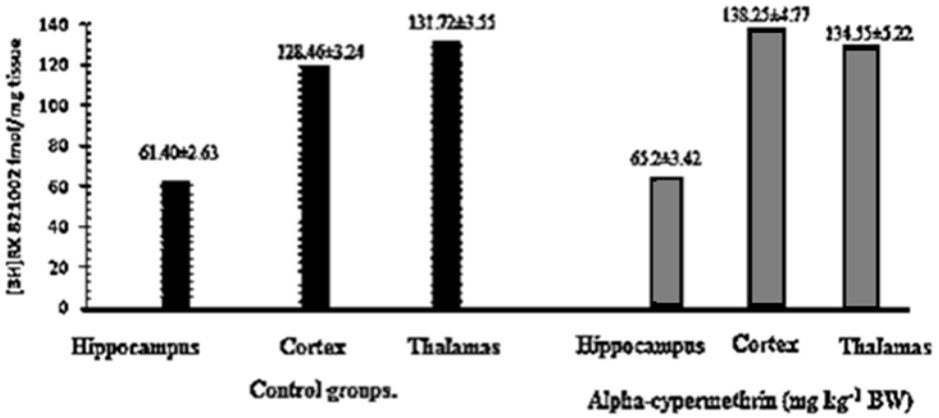

Figure 3 Effect of sub-lethal oral dose of alpha-cypermethrin on the binding [3H] RX821002 to α2-adrenergic receptors in hippocampus, cortex and thalamus of the rat brain (2 hours post treatment).

3.1. Concentrations of alpha-cypermethrin single oral sub-lethal dose in blood plasma of rats (25 mg kg-1 B W)

As shown in Table, 1 and Fig.1, Alpha-cypermethrin 25 mg kg^{-1} B W concentrations in blood plasma of rat after 1, 2, 3 and 24 hr. were 0.416±0.158; 0.233±0.058; 0.162±0.058 and 0.257±0.158, respectively.

It is likely that the pattern of concentration in lipid-rich tissues is due to the high lipid solubility of pyrethroid compounds. Since metabolism of pyrethroids results in products that are more water-soluble than the parent compounds, it is likely that the metabolites are less able to cross the blood-brain barrier, unless there are facilitated mechanisms for transport of pyrethroid metabolites that have not yet been characterized.

However, these compounds are highly toxic to fish and other lower aquatic organisms [11], and their widespread use has led to toxic effects in plants, animals, and human beings.

No information was located regarding the transport of pyrethroid compounds in blood. Pyrethroids are distributed to nearly all tissues and are concentrated in tissues with high lipid contents, such as fat and nerve tissue [12,13].

3.2. Effects on the extracellular Serotonin levels in rat ventral hippocampus

Extracellular serotonin levels % (f mol/mg tissue) in rat ventral hippocampus at 25 mg kg^{-1} B.W. after 1, 2, 3 and 24 hr. were 105±4, 80 ±7, 80 ±4, 70 ±4, respectively. Alpha-cypermethrin administered acutely, at 2 hr.,3hr. and 24 hr. after dosing, revealed significant *(P < 0.05)* increase in extracellular serotonin levels % (f mol/mg tissue) in rat ventral hippocampus, cortex and cortex as shown in Table 3 and Fig.3.

Alpha-cypermethrin acts as serotonin reuptake inhibitors (SRIs) in rat ventral hippocampus, cortex and thalamus at the presynaptic alpha-2 adrenergic receptors [14]. Alpha-cypermethrin administered , at 1 hr. and up to 24 hr. after dosing, revealed significant increase in α_2-adrenergic receptors in all brain regions (ventral hippocampus ,cortex and cortex), as evidenced by significant *(P < 0.05)* increased number of [^{3}H]RX821002 (binding sites in such brain regions)].

Our findings revealed that, Alpha-cypermethrin acts as nor-epinephrine reuptake inhibitors (NRIs) in rat ventral hippocampus, cortex and thalamus at the presynaptic alpha-2 adrenergic receptors [14]. The mechanism by which Alpha-cypermethrin works is not clear (it might prevent the reuptake of norepinephrine by the presynaptic neuron). Alpha-cypermethrin administered , at 2 hr. and up to 24 hr. after dosing, revealed significant increase in α_2-adrenergic receptors in all brain regions (ventral hippocampus and cortex), as evidenced by significant *(P < 0.05)* increased number of [^{3}H]RX821002 (binding sites in the hippocampus and cortex)] [15].

3.3. Effects on the extracellular Noradrenalin levels in rat ventral hippocampus.

Extracellular NA levels % (f mol/mg tissue) in rat ventral hippocampus at 25 mg kg^{-1} B.W. after 1, 2, 3 and 24 hr. were 300±10, 450 ±14, 400±12, 400±11, respectively as shown in Table, 2and Fig.2. Alpha-cypermethrin administered acutely, at 2 hr.,3hr. and 24 hr. after dosing, revealed significant *(P < 0.05)* increase in extracellular NA levels % (f mol/mg tissue) in rat ventral hippocampus and cortex.

Our findings revealed that, Alpha-cypermethrin acts as nor-epinephrine reuptake inhibitors (NRIs) in rat ventral hippocampus, cortex and thalamus at the presynaptic alpha-2 adrenergic receptors [14]. The mechanism by which Alpha-cypermethrin works is not clear (it might prevent the reuptake of norepinephrine by the presynaptic neuron). Alpha-cypermethrin administered , at 2 hr. and up to 24 hr. after dosing, revealed significant increase in α_2-adrenergic receptors in all brain regions (ventral hippocampus and cortex), as evidenced by significant *(P < 0.05)* increased number of [^{3}H]RX821002 binding sites in the hippocampus and cortex [15].

Cypermethrin has other effects on aminobutyric acid receptor. It inhibits excitement and convulsions. In addition, it prevents the absorption of calcium (uptake) from the nerves and inhibits monoamine oxidase, an enzyme that breaks down neurotransmitters [16]. Cypermethrin also affects an enzyme not directly related to the nervous system, adenosine triphosphatase. It is involved in the production of cellular energy, the transfer of metal atoms, and muscle cramps and contractions.

Pyrethroid-dependent neurotransmitter release from presynaptic nerve terminals in the brain was first documented in rats treated with deltamethrin [17]. Treatment of deltamethrin resulted in a significant reduction in acetylcholine levels in the entire brain, and most importantly in the cerebellum. In contrast, cismethrin did not produce any significant reduction in acetylcholine levels [17].

Other effects of pyrethroids (deltamethrin and cypermethrin) in the cerebellum include increases in cyclic guanosine monophosphate levels [17,18,19,20] and enhanced calcium-dependent neurotransmitter release by fenvalerate from rabbit striatal slices but not from hippocampal slices.

Pyrethroids also increased the levels of some amino acid neurotransmitters and metabolites of monoamine neurotransmitters in the brain. The release of neurotransmitters is a highly organized event dependent on external calcium influx via voltage-sensitive calcium channels associated with the plasma membrane of the presynaptic nerve terminals [21].

The histopathological changes of brain (Fig.4 a, b, c and d) in the acute sub-lethal-dose group (25 mg kg $^{-1}$ BW) were congestion, slight neuronal vacuolation, hemorrhages, extracellular brain edema and focal glial cells infiltrations (Gliosis) [22.23].

In cypermethrin-intoxicated rats, pyknosis of the Purkinje cells and disappearance of some of the cells in the cerebellum was observed by Luty *et al*. [24]. Pyknosis of neurons was observed cypermethrin-intoxication in rats. Congestion and degenerative changes in the brain were reported in calves and rats, respectively. Manna *et al*., (2004a and b) [23,25] reported congestion and hemorrhages in the brain of rats intoxicated with single and repeated doses of cypermethrin. In cypermethrin-intoxicated rats, Grewal *et al*. [26] observed neuronal degeneration and necrosis in the cerebrum. Pesticide exposure is recognized as a risk factor for Alzheimer's disease (AD). The signs of AD-like pathology upon exposure to cypermethrin, it reported that, impairs neurodevelopment. Cypermethrin (10 and 25 mg/kg^{-1}) increased the key proteins of AD, amyloid beta (Aβ), and phospho-tau, in frontal cortex and hippocampus as early as postnatal day 45 [27].

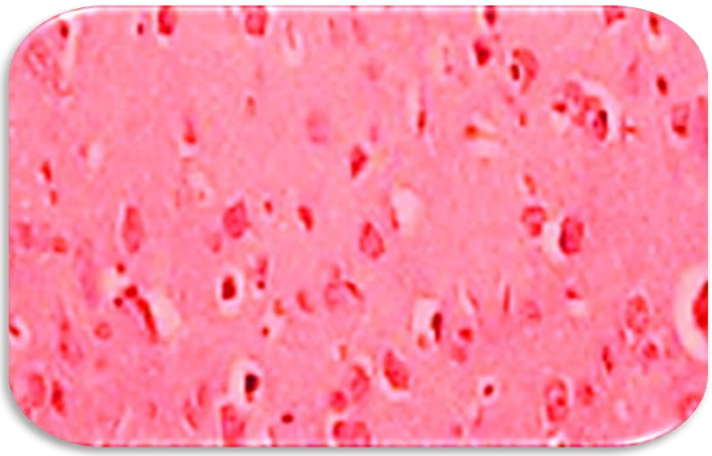

Figure 4a Brain of rat showing normal histological structures (H&E, X 400).

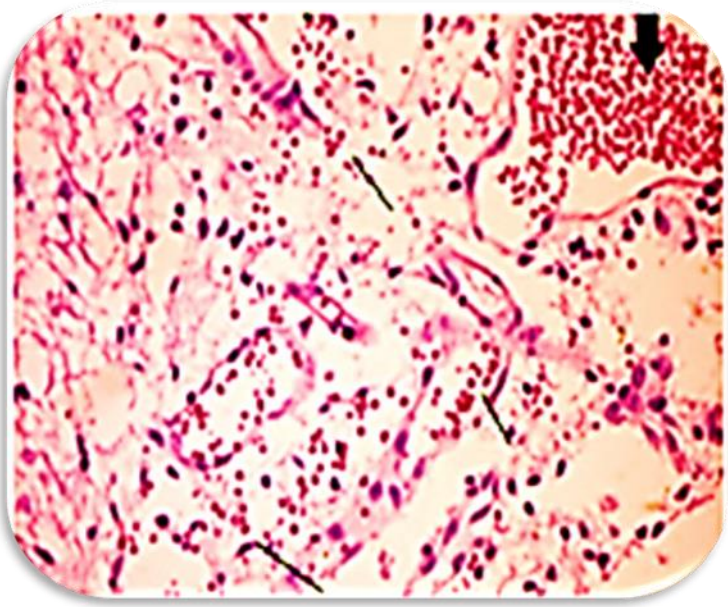

Figure 4b Brain of rat showing Severe edema, congestion (arrowhead) and hemorrhage (arrows) (H&E, X200).

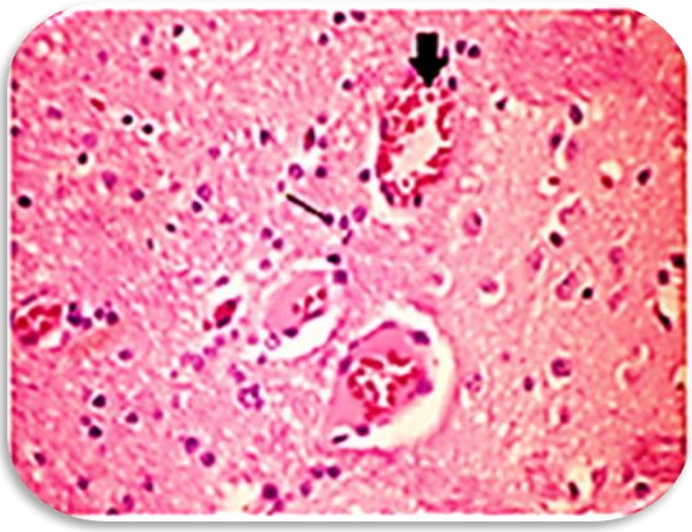

Figure 4c Brain of rat showing congestion and gliosis (arrows) (H&E,X200).

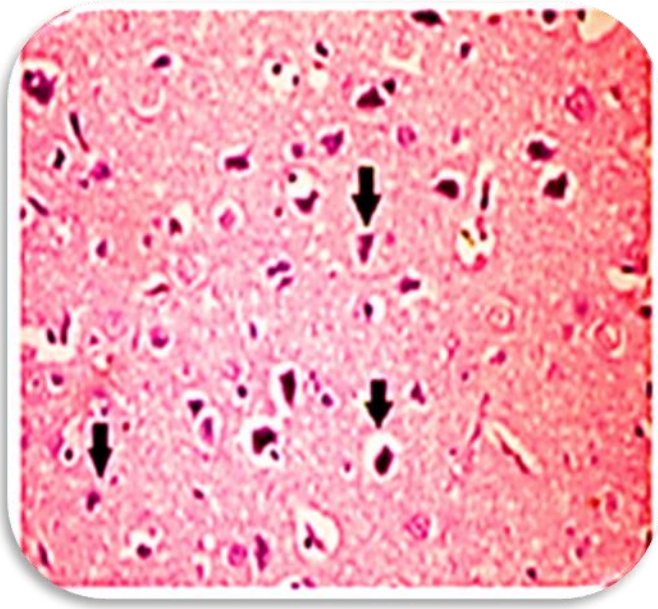

Figure 4d Brain of rat showing degenerative neurons (arrows) (H&E, X200).

4. Conclusion

A single sub-acute lethal -oral dose of Alpha-Cypermethrin (25 mg kg $^{-1}$) in rat were result in lesions of brain tissues: gliosis, perineuronal vacuolation, perivascular vacuolation, and neuronal degeneration. In Addition to, increasing of the extracellular serotonin and noradrenalin levels in ventral hippocampus, cortex and thalamus. Alpha-Cypermethrin acts as nor-epinephrine reuptake inhibitors (NRIs) and serotonin reuptake stimulator in rat brain. These findings are of interest, since elderly humans experiencing transient ischemic attacks are chronically exposed to cypermethrin, used for household pest control or via contaminated fruits, vegetables and drinking water.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of ethical approval

All efforts were made to minimize the number of animals and their suffering in this study through following the guidelines released by Cairo university policy on Animal Care and Use.

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