Neurotoxicity of Cypermethrin on Rat Cerebral Cortex and the Alleviated effect of Hydroxytyrosol

Original Article

Amira Ebrahim Alsemeh, Mohamed A. M. AbdelRahman, Fatma A. Mahmoud, Eman Ramadan Abd El Fattah

Departments of Anatomy, Faculty of Medicine, Zagazig University, Egypt.

ABSTRACT

Cypermethrin (CYP) is a synthetic pyrethroid. It crosses the blood-brain barrier in pests and creates neurotoxicity in the CNS. Hydroxytyrosol (HT) is considered a powerful natural antioxidant, it is found in olive leaf, olive pulp and olive oil. Hydroxytyrosol is the only phenol that is capable to pass the blood-brain barrier and permits it to involve free radicals throughout the CNS. We designed this study to explore the possible protective role of HT against the neurotoxic effect induced by CYP. Thirty male adult albino rats were divided into three groups. Control group; subdivided into: control, corn oil, HT groups, CYP treated and CYP, CYP+ HT treated group. Treatments were given by gavage for 14 days. The cerebrum tissue was prepared for histopathological examination and immunohistochemical detection of caspase 3 and GFAP. We also assessed the level of GAPA and ACH in the tissue and the oxidative enzymatic and the lipid perioxidation profile. We observed that the concomitant use of HT with the CYP diminished the signs of hyper excitability induced by the CYP which confirmed by significant decrease in the level of GABA in cerebrum tissue. HT counteracted the histopathological changes induced by CYP and significantly downregulated the caspase -3 and GFAP expression in cerebrum tissue. A rescue of the oxidative enzymatic activity, lipid peroxidation profiles was also evident in the CYP+HT-treated group. We concluded that HT exhibited substantial antioxidant and anti-apoptotic impacts on the cerebral cortex cytomorphological alterations and apoptosis produced by CYP in cerebrum tissue.

Key Words: Cypermethrin, GABA, GPX, Hydroxytyrosol, MDA.

Revised: 19 December 2020, **Accepted:** 17 May 2021.

Corresponding Author: Amira Ebrahim Alsemeh, MD, Department of Anatomy, Faculty of Medicine, Zagazig University, Egypt, **Tel.:** 0552378971, **E-mail:** dr_amira_2008@yahoo.com

ISSN:2536-9172, June 2020, Vol. 4, No. 2

INTRODUCTION

Cypermethrin is a type II pyrethroid that widely used as insecticides in houses and agriculture. It is also used in medical field as topical treatment of scabies and head lice. In tropical countries, it is used for malaria control, the bed nets are soaked in CYP to prevent mosquito bites[1,2].

CYP is considered as the most harmless pesticide used worldwide 12, but sign has shown that CYP could induce numerous toxic effects including developmental neurotoxicity, oxidative stress and apoptosis[3].

The lipophilia of CYP favored its absorption through the skin, gastrointestinal tract, and respiratory tract to be more disseminated into internal tissues which is rich in lipid, including body fat, parts of the central and peripheral nervous system[4].

Synthetic pyrethroids pesticides are toxic to the central nervous system in acute intoxication and this mainly mediated through hyper-excitation of the CNS. CYP crosses the blood-brain barrier in pests and creates neurotoxicity and motor deficits[5].

CYP produces neurotoxicity by making a long-lasting prolongation in NA++ channels permeability of the neuronal membrane through its excitation which leading to repetitive nerve impulses in the central nervous system. These impulses result in oxidative stress producing extreme reactive oxygen species (ROS)[2,6].

3,4-dihydroxyphenylethanol [HT] is a phenolic compound which is found in in olive leaf, olive pulp and olive oil. It is one of the powerful antioxidants in the nature[7,8]. It is the only phenol that passes the blood-brain barrier to involve free radicals throughout the CNS protecting blood lipids from oxidative damage. It is a metabolite of the neurotransmitter dopamine, so it has a role in protection of nervous system[9,10].

HT has other extraordinary characteristics that contribute to its efficacy. It is quickly absorbed into the circulation and tissues, where it can achieve its free radical scavenging responsibilities. It has

 DOI: 10.21608/jmh.2021.54223.1087

an ORAC value (Oxygen Radical Absorbance Capacity - its capability to involve cell-damaging free radicals) [11]. This work was designed to explore the possible protective role of HT against the neurotoxic effect induced by CYP.

MATERIALS AND METHODS

2.1. Chemicals

-Cypermethrin: CP 98% produced by Jiangsu Yangnong Chemical Group Co L.T.D., China.

-Hydroxytyrosol: was purchased from ProHealth, Inc. Carpinteria, Santa Barbara County, California, USA.

-Corn oil: was brought from commercial sources.

-Distilled water: was brought from Kemecta Company.

2.2. Experimental animals:

This study was carried out on thirty adult male albino rats Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt) weighing 200-250 gm for each. They were housed under a controlled temperature (22 °C) and humidity (50 ± 10%) at 12 h light/dark cycle with food (regular chow) and water supplied ad libitum. Experimental design and treatment protocols were approved by the Ethical Committee for Animal Handling at Zagazig University (ECAH ZU),

2.3. Experimental design:

The rats were divided into 3 groups as follows:

Control group: this included 18 rats equally divided into 3subgroups:

Negative control group: This group was not given any treatment and was given water ad-libitium and balanced diet for 14 days.

Corn oil group: rats were given 1ml/kg/day of corn oil (solvent of CYP) by gavage for 14 days.

HT group: rats were given 50ml/kg/day of HT dissolved in 1ml distilled water by gavage for 14 days[12].

Cypermethrin (CYP)-treated group: this group included 6 rats, were treated with CYP (20mg/kg/day) dissolved in 1 ml corn oil by gavage for 14 days[13].

CYP + HT-treated group: this group included 6 rats, these rats were treated with20 mg/kg/day of CYP along with 50 mg/kg/d of HT for 14 days

After 24 hours from the last administration, all animals were anesthetized by intra-peritoneal injection of thiopental 75 mg/kg[14]. Afterward, careful craniotomy was done, and the vault of each animal cranium was removed. After removing the meninges from brain, it was smoothly lifted out of the skull. Then the cerebrum was gently divided into two halves. One half was wrapped with aluminium foil and kept frozen at -80 °C until used for biochemical studies. The other half was fixed in 10% formol saline for 48 hours for histo-pathological studies.

2.4. Biochemical studies:

Specimens were minced and homogenized (10% W/V) separately in ice-cold saline, sucrose buffer (0.25 M sucrose, 1 mM EDTA and 0.05 M Tris–HCl, pH 7.4) in a Thomas Sci. Co. glass-type homogenizer (Teflon pestle). The homogenization of tissues was carried out in Teflon-glass homogenizer with a buffer containing 1.15% KCl to obtain 1:10 (W/V) whole homogenate. Homogenates were centrifuged at 3000 rpm (+4 °C) for 15 min to determine Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) while, homogenates were centrifuged at 5000 rpm for 50 min to measure gamma-Aminobuyric acid (GABA) and Acetylcholine (Ache) activity.

MDA is detected as an end product of lipid peroxidation MDA was assayed according to the method proposed by Ohkawa *et al*.[15]. SOD activity in brain was carried out according to the method of Fridovich[16]. Tissues GPx enzyme activity was measured by the method of Chiu *et al*.[17].

According to the method described by[18], we determined

GABA levels in the cerebral cortex by using high-performance liquid chromatography with ultraviolet detectors (Waters China, Milford, Massachusetts, USA),

Ache was extracted with 1 ml 0.5% sodium tetraphenylboron in 85% toluene-15% acetonitrile. After centrifugation, aliquots of 0.8 ml were used for tritium quantification with a scintillation counter (Wallac system 1409, Perkin Elmer).[19].

2.5. Tissue preparation for Histopathological and Immunohistochemical

Studies for light microscopic examination

Specimens of cerebral cortex were fixed with4% paraformaldehyde in 0.1M phosphate buffer overnight at 4°C. After fixation, the cerebrum tissues were dehydrated in graded ethanol, implanted in paraffin and partitioned

to obtain paraffin sections (3-µmthick). Some paraffin sections were deparaffinized and hydrated, followed by staining with hematoxylin and eosin stain (H&E)[20]., The tissues submitted to the light microscopic examination.

For immunohistochemical analysis, deparaffinized and hydrated sections were incubated for 20 min at 105°C in Citrate buffer (pH 6.0) for antigen retrieval of the following proteins rabbit polyclonal

1- Cystiene aspartic acid specific protease-3 (caspase- 3) is an effector marker for apoptosis increased in neural diseases. Rabbit monoclonal antibody of IgG type against caspase 3 (dilution 1:100) delivered from Lab Vision Laboratories. Apoptotic cells observed with yellow to brown discoloration.

2- Glial fibrillary acidic protein (GFAP) for Astroglia. The positive results were indicated by brown coloration of the cell membrane and cytoplasm of the astrocytes. This stain is considered specific for the intermediate filaments fibrillary acidic protein found in astrocytes and it is not found in nerve cells and even other types of glial cells as oligodendrocytes or microglial[20,21].

2.6. Preparation for Transmission electron microscope:

Specimens of cerebral cortex were immediately fixed in 2.5% glutaraldehyde buffered with 0.1 mol/L phosphate buffer at PH 7.4 for two h at 4ºC, post fixed in 1% osmium tetraoxide in the same buffer for 1 h at 4ºC. The specimens were embedded in flat capsules then polymerized at 60Cº for twenty-four h. ultrathin sections were obtained using lecia ultracut UCT, stained with uranyl acetate and lead citrate[22] The tissues submitted to the electron microscopic examination in electron microscope unit, Faculty of Medicine, Mansura University.

Statistical analysis:

Data were analyzed by Statistical Package of Social Science (SPSS), software version 24.0 **(SPSS Inc.2016)**. Continuous data were presented as the Mean (SD). Normality was checked by Shapiro test. Homogeneity of variances was checked by Leven's test.

A one-way ANOVA: it is used to determine whether there are any significant differences between the means of two or more independent (unrelated) groups on a continuous dependent variable. Furthermore, post-hoc tests (Tukey test if equal variances are assumed; Games-Howell test if equal variances are not assumed) were performed.

Tukey post hoc test: it is used for multiple comparisons between groups following ANOVA test.

Paired-samples t-test (dependent t-test): it is used to compare the means between two related groups.

All statistical comparisons were two-tailed.

Significance level:

P-value <.*05* indicates significant, *P* <.*01* indicates highly significant difference, *P* <.*001* indicates very highly significant difference while, *P* ≥.*05* indicates non-significant difference[23].

$$\text{Relative brain weight} = \frac{\text{Absolute brain weight (g)}}{\text{Body weight of rat on the day of sacrifice (g)}} \times 100$$ [24]

RESULTS

4.1. Sign of toxicity and body weight measurements:

During the experimental period, there were signs of neurotoxicity in rats treated with CYP as irritability, erected furs, suspended tail, itching, startle response, and crawling gait (a result of splaying of the hind limbs). Also, there was loss of weight, decrease in feeding and increase in the bowl movement. At the ninth and eleventh days after administration of CYP, two rats died which were replaced by other rats. However, rats treated with CYP and HT revealed delayed appearance of irritability, erected furs, and startle response. The rats were normal in their gait, feeding and bowel movement. There is no mortality in this group (Figure.1).

The body weights were statistically analyzed and non-statistically significant change in mean body weight among the different studied groups at the day zero of experiment was observed, (*P*=.*93*). In contrast, there were statistically significant differences in the mean body weight among different studied groups at the last day of the experiment (*P*<.*001*). Tukey post hoc test revealed no statistically significant differences among negative control, corn oil and HT groups (*P*>.*05*). CYP-treated and CYP+HT-treated groups had statistically significant lower values of the mean body weights compared with control groups (*P*<.*05*). But CYP+HT -treated group had statistically significant higher values of the mean body weights compared with CYP-treated group (*P*<.*05*).

The mean body weights were significantly increased at the last day of the experiment compared with zero day of the experiment in the negative control, corn oil and HT groups (*P*<.*05*).

The mean body weights were significantly decreased at the last day of the experiment compared with zero day in CYP-treated group (*P<.05*). But in CYP+HT-treated group, the mean body weights were statistically significant similar at the last day of the experiment compared with the zero day (*P>.05*).This is illustrated in (Table.1 and Figure 2).

Also the relative brain weight were analyzed as there were no statistically significant differences in mean relative brain weights among different groups of the study (*P<.05*). Tukey post hoc test revealed non statistically significant differences among negative control, corn oil and HT, CYP+HT-treated groups (*P>.05*). CYP-treated group had statistically significant lower level of the mean relative brain weights compared with control groups and CYP+HT-treated groups (*P<0.05*) as in (Table.2 and Figure 3).

4.2. Biochemical results

Lipid peroxidation and antioxidant enzyme activity

Regarding the mean brain lipid perioxidation biomarker level (MDA),Tukeypost hoc test revealed non statistically significant differences among negative control, corn oil and HT groups (*P>.05*). CYP-treated and CYP+HT-treated groups had statistically significant higher levels of brain MDA compared with control groups (*P<.05*). But CYP+HT-treated group had a statistically significant lower level of the mean brain MDA compared with CYP-treated rats (*P<.05*).This was presented in (Table 3 and Figure 6).

Regarding mean brain antioxidant biomarkers levels (GPX, and SOD), Tukey post hoc test revealed non statistically significant differences among negative control, corn oil and HT groups (*P>.05*). CYP-treated and CYP+HT-treated groups had statistically significant lower mean brain antioxidant biomarkers levels (GPX, and SOD) compared with control groups (*P<.05*). But, CYP+HT -treated group had statistically significant higher mean brain antioxidant biomarkers levels compared with CYP-treated group (*P<.05*), this was illustrated in (Table 3and Figures 4,5).

Regarding to the brain neurotransmitters (GABA and Ache), Tukey post hoc test revealed non statistically significant differences in mean brain GABA concentration among negative control, corn oil and HT groups (*P>.05*). CYP-treated and CYP+HT-treated groups had a statistically significant lower mean brain GABA concentration compared with control groups (*P<.05*). But, CYP+HT-treated rats had a statistically significant higher mean brain GABA concentration compared with CYP-treated rats (*P<.05*). There were statistically significant differences in mean brain GABA concentration (*P<.05*) but a non-statistically significant difference in mean brain Ache concentration among different groups of the study (*P>.05*). This was illustrated in (Table 4 and Figures 7,8).

4.3. Histopathological results:

4.3.1. Light microscopic results:

4.3.1.1. Hematoxylin and eosin (H&E):

Hematoxylin and eosin stained sections of all control subgroups revealed similar histological structure of the cerebral cortex, they considered as one group. They showed the well-known normal histological structure of the cerebral cortex, which was arranged in six successive layers. The outer molecular was the first layer underneath the meninges and consisted of neuropil and few cell bodies of neurons. The external granular contained many granule cells. The next one was the external pyramidal layer that contained small pyramidal cells. The internal granular layer was the fourth one and composed of granule cells. The fifth one was internal pyramidal that contained cell bodies of large pyramidal cells. The sixth layer is the most inner one was the multiform layer which was dominated by elongated spindle shaped cells (Fig.5a). In the outer layers, The granule cells appeared rounded in shape and showed pale rounded open face nuclei with prominent nu- cleoli surrounded by little cytoplasm. The pyramidal cells were small and conical in shape. Glial cells appeared small in size with small well demarcated nuclei and others with perinuclear space. The acidophilic background was the neuropil which was a mat of neuronal and glial cell processes and blood vessels with a narrow perivascular space (Fig.5b). In the inner layers, granule cells had pale rounded open face nuclei with prominent nucleoli surrounded by little cytoplasm. The pyramidal cells were large and had vesicular nuclei and basophilic cytoplasm. The acidophilic neuropil contained glial cells with small well demarcated nuclei and others with perinuclear space and blood vessels with a narrow perivascular space (Fig.5c).

H & E-stained sections of rat's cerebral cortex of CYP-treated group showed histological changes in all layers of the cortex in comparison to the control group. There was marked separation of pia matter with disorganization of the six layers of the cerebral cortex and invasive blood vessels can be seen (Figs. 6 a,b). The outer layers showed most of cortical neurons were distorted in shape. Most of Pyramidal cells were affected with long process, shrunken dark stained nuclei and surrounded by vacuolated pale halo most probably apoptotic cells. Neuroglia cells with darkly stained nuclei surrounded by white space were seen. The neuropil was highly vacuolated with congested dilated blood vessels that surrounded by wide perivascular spaces (Fig. 6c). The inner layers showed affected pyramidal cells as in the outer layers. The neuropil was vacuolated & contained neuroglia cells and congested dilated blood vessels that surrounded by wide perivascular spaces (Fig. 6d). Cellular infiltration (fig. 6e) and hemorrhage were seen in some areas (Fig. 6f).

H & E stained sections of rat's cerebral cortex of CYP + HT-treated group showed moderate improvement in nerve cells in many areas and moderate improvement in organization of six layers. The pia matter was slightly separated (Fig. 7a). The outer layers showed multiple normal small conical-shaped pyramidal cells and few affected pyramidal cells with dark stained nuclei. The neuropil was still vacuolated & contains neuroglia and blood vessel that are surrounded by wide perivascular space (Fig. 7b). The inner layers showed normal large pyramidal cells with vesicular nuclei and basophilic cytoplasm. Others few pyramidal cells were affected. Normal Granule cells with pale rounded open face nuclei with prominent nucleoli surrounded by little cytoplasm were seen. The neuropil is less vacuolated & contains neuroglial cells. (Fig. 7c).

4.3.1.2. Immunohistochemical results

4.3.1.2.1. Caspase -3 immunostaining results

The cerebral cortex was immune-histochemical stained with anti-caspase 3 antibody to clarify the localization of apoptotic neurons in all experimental groups. The immune positive reaction was localized in the cytoplasm of apoptotic neurons. In control group, neurons revealed negative immunoreaction (Fig 8.a). However, in the CYP treated group, abundant caspase immune-positive neurons were detected (Fig 8.b). Whereas fewer caspase-3 immuno-positive neurons were observed in the cerebral cortex of the CYP + HT treated group (Fig 8.c).

4.3.1.2.2. GFAP immunostaining results

The cerebral cortex was immunohistochemically stained with the anti-GFAP antibody to show the response of astrocytes to the neural damage in the different studied groups. Control group showing GFAP positive staining in the cytoplasm of astrocytes and their processes. They appeared small and few in number with short thin few processes (Fig.9. a). However, in CYP treated group there were abundant GFAP positive staining of the cytoplasm and the processes of astrocytes. They were apparently increased in number and appeared larger with multiple long thick processes (Fig.9. b). In CYP+HT treated group few astrocytes appeared large with long thick processes and others were small in size with few thin short ramified processes (Fig.9. c).

4.3.1.3. Toluidine blue stain results

Toluidine blue semithin sections of control groups showed normal neurons with large open-faced nuclei surrounded by pale cytoplasm containing basophilic Nissl's granules. Glial cell with sharply rounded demarcated nuclei can be observed within the intact neuropil (Fig.10a). In CYP treated group, deeply stained cortical neurons are seen. Normal neurons were also noticed. Neuropil are vacuolated and blood vessels were surrounded with a wide perivascular area (Fig.10b). The cerebral cortex of CYP+ HT treated group showed that the most neurons were normal and few neurons with deeply stained nuclei. Blood vessels with narrow perivascular space in intact neuropil can be noticed (Fig10.c).

4.4. Electron microscopic results

In control group, cortical neurons nuclei were euchromatic. The cytoplasm was rich in organelles as numerous mitochondria, rough endoplasmic reticulum (rER) and Nissl's granules (Fig. 11.a). Astrocytes had large well- demarcated euchromatic nuclei and surrounded by a narrow electron lucent cytoplasm with few cytoplasmic organelles such as glycogen granules and ribosomes (Fig. 11.b). In contrast, CYP treated group exhibited shrunken cortical neurons with heterochromatic nucleus. The nuclear envelop was irregular and characterized by the presence of indentations. The Cytoplasm was characterized by the dilated cisterna of the rER , few scattered rER, deformed mitochondria, few spread Nissl's granules, spread of polysomes (pl) , and electron-dense bodies (Fig. 12.a) Astrocytes had rounded heterochromatic shrunken nuclei and wide irregular electron lucent cytoplasm. It had swollen mitochondria, many electron-dense bodies with dilated cisterna of the rER (Fig. 12.b). However, co-adminstration of the HT in concomitant with the CYP restore the most normal appearance of cortical neurons (Fig 13.a) and astrocytes (Fig.13.b).

Table 1: Effects of CYP, CYP+HT on the body weight of the different studied groups.

Groups / Body weight (g)	Timing: Baseline	Timing: At the end	Paired t test	***P-value***
Negative control Mean±SD	216.3±10.6	225.5±8.1[a]	t=4.9	.005
Corn oil) Meanc± SD	218±9.2	227±7.7[a]	t=3.5	.018
HT Mean± SD	218.8±9.1	225.8±7.4[a]	t=4.3	.008
CYP Treated Mean± SD	218.1±7.8	155.1±13.1[b]	t=11.7	<.001
CYP+ HT Mean± SD	216.7±7.5	201.6±19.1[c]	t=2.1	.07
ANOVA	F=0.099	F=45.3		
P-value	.93	<.001		

CYP, Cypermethrin, HT, Hydroxytyrosol, means in a column without a common superscript letter differ (*P<0.05*) by post hoc Tukey test for multiple comparisons between groups, n=6 in control groups and 9 in treated groups.

Table 2: Effects of CYP, CYP+HT on relative brain weight in the different studied groups**.**

Variables	Negative Control n=6	Corn oil n=6	HT n=6	CYP Treated n=9	CYP + HT n=9	ANOVA	***P-value***
Relative brain weight (g brain weight/100 g body weight)						F=15.1	***<.001***
Mean±SD	0.75±0.06[a]	0.76±0.06[a]	0.78±0.07[a]	0.50±0.07[b]	0.67±0.12[a]		

CYP, Cypermethrine, HT, Hydroxytyrosol, means in a row without a common superscript letter differ (*P<0.05*) by Tukey post hoc test for multiple comparisons between groups.

Table 3: Effects of cypermethrin, cypermethrin plus hydroxytyrosol on brain oxidative stress biomarkers in the experimental rats.

Variables	Negative Control n=6	Corn oil n=6	HT n=6	CYP Treated n=9	CYP + HT Treated n=9	ANOVA	*P-value*
GPX (U/g tissue) Mean±SD	50.1±0.8[a]	50.8±2.6[a]	51.5±3.1[a]	20.1±1.2[b]	39.8±1.1[c]	F=413.2	*<.001*
Mean±SD	5.3±0.48[a]	5.1±0.35[a]	5.1±0.30[a]	2±0.1[b]	3.8±0.76[c]		
SOD (U/g tissue) Mean±SD	5.1±0.3[a]	5.3±0.5[a]	5.2±0.4[a]	2.1±0.3[b]	3.9±0.3[c]	F=114.6	*<.001*
MDA (nmol/g tissue) Mean±SD	20.03±0.13[a]	19.6±0.89[a]	19.4±1.2[a]	69.8±4.1[b]	51.3±2.6[c]	F=643.0	*<.001*

CYP, Cypermethrine ,HT, Hydroxytyrosol, GPX, glutathione peroxidase, SOD, superoxide dismutase and MDA, Malondialdehyde means in a row without a common superscript letter differ (*P<0.05*) by Tukey post hoc test for multiple comparisons between groups.

Table 4: Effects of cypermethrin, cypermethrin plus hydroxytyrosol on brain neurotransmitters in the experimental rats.

Variables	Negative Control n=6	Corn oil n=6	HT n=6	CYP Treated n=9	CYP + HT Treated n=9	ANOVA	*P-value*
GABA (pg/ml) Mean±SD	105.2±7.2[a]	102.9±7.1[a]	102.4±6.7[a]	50.3±4.7[b]	80.0±7.2[c]	F=100.6	<.001
AchE (pg/ml) Mean±SD	48.7±6.7	48.2±7.8	48.9±6.7	44.8±7.2	46.1±8.7	F=0.43	.79

CYP, Cypermethrine, HT, Hydroxytyrosol, Ach, Acetylcholine GABA, gamma-Aminobutyric acidmeans in a row without a common superscript letter differ (*P<0.05*) by post hoc Tukey test for multiple comparisons between groups.

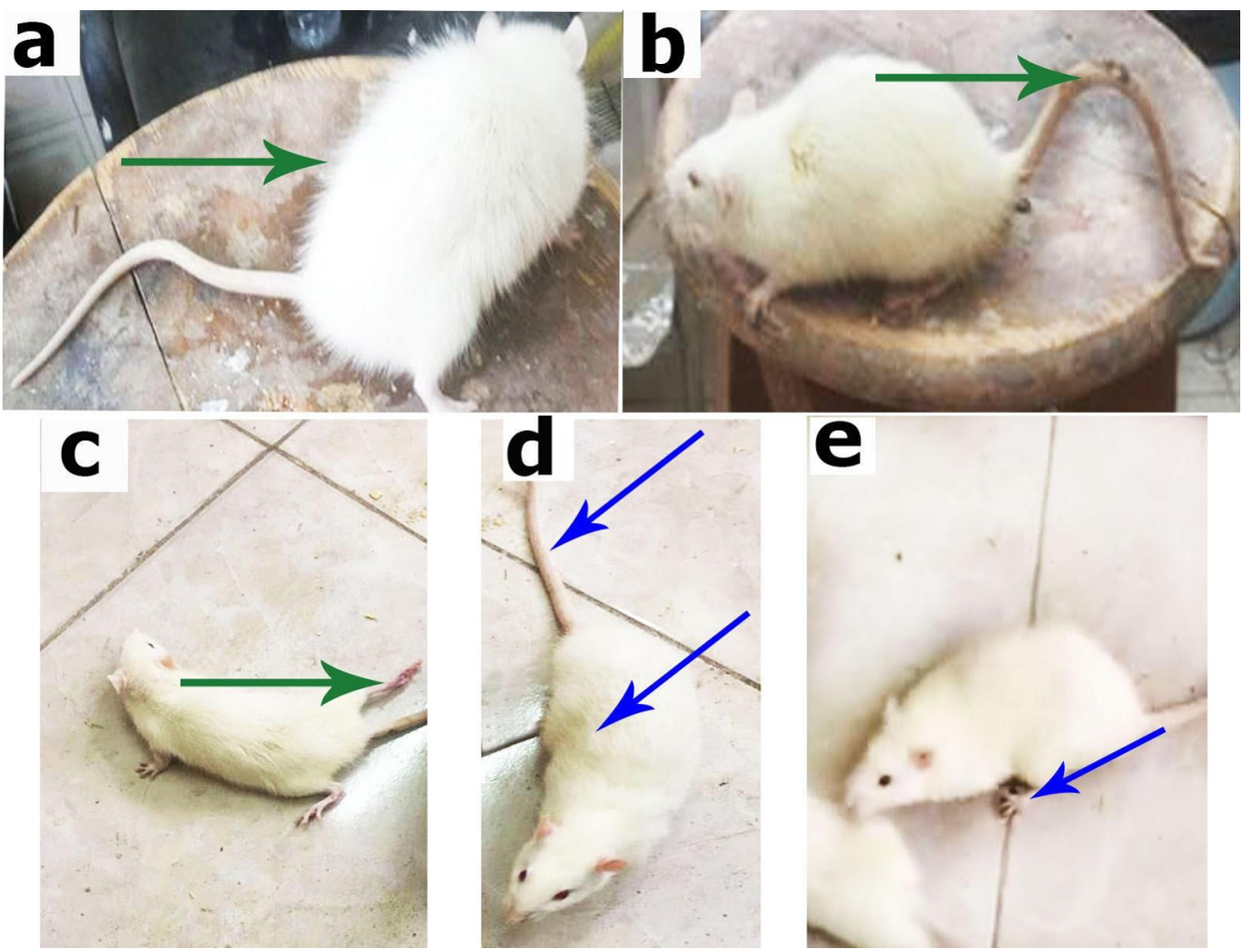

Fig.1. a, b, c: Photographs of rats treated with CYP showing signs of neurotoxicity (green arrow). a) erected fur, b) suspended tail, c) splaying hind limb. d, e: Photographs of rats treated with CYP+HT showing delayed appearance of neurotoxicity signs (blue arrow). d) normal hair appearance & normal tail, e) normal hind limb.

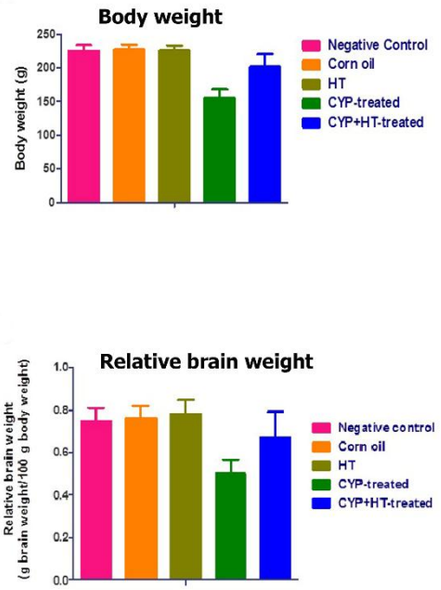

Fig. 2: Bar chart showing effects of CYP, CYP+HT on body weight and relative brain weight in the different studied groups.

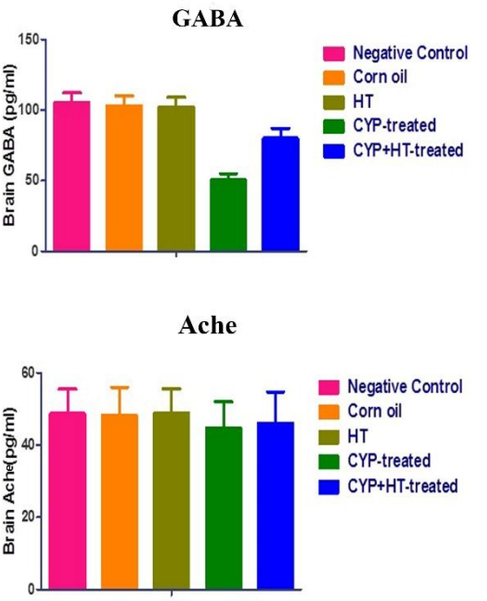

Fig. 4: Bar chart showing effects of CYP, CYP+HT on brain GABA and Ache concentration in the different studied groups. Data are Mean ±SD.

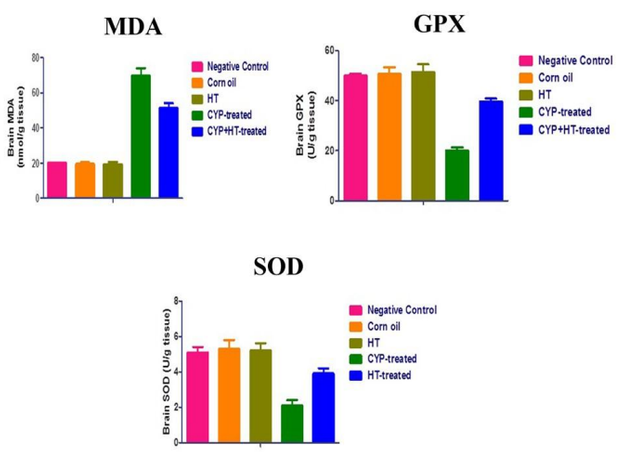

Fig. 3: Bar chart showing effects of CYP and CYP+HT on brain MDA, GPX and SOD levels in rat cerebrum of the different studied groups. Data are Mean±SD.

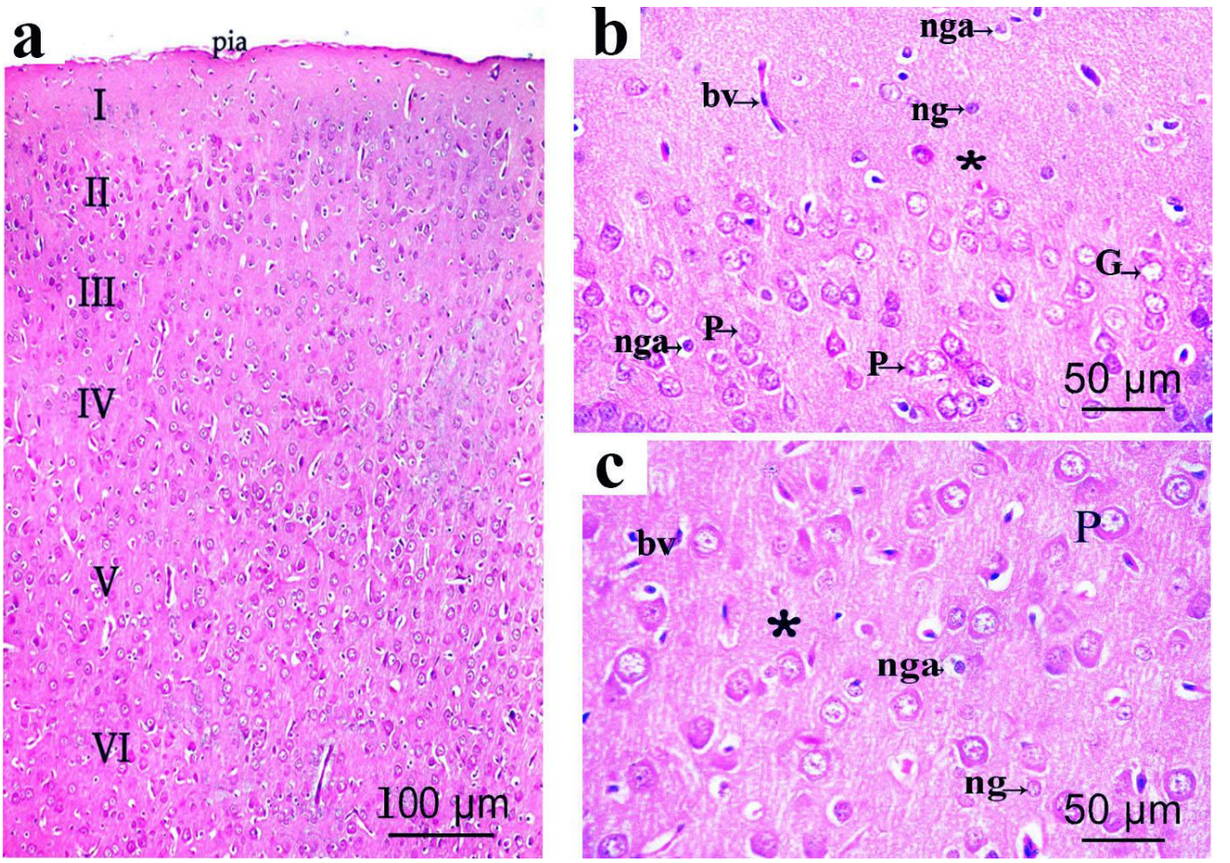

Fig. 5: photomicrographs of a section in a rat's cerebral cortex of the control group showing a) normally attached pia matter (pia) and normal organization of the six layers of the cerebral cortex from superficial to deep: molecular layer (I), outer granular layer (II), outer pyramidal layer (III), inner granular layer (IV), inner pyramidal layer (V) and the multiform layer (VI).b) Higher magnification of the outer layers showing the granule cells (G) with pale open face nucleus and little cytoplasm. The outer pyramidal layer contains small conical-shaped pyramidal cells (P) with basophilic cytoplasm. The acidophilic neuropil (*) contains blood vessels with narrow perivascular spaces (bv) and normal neuroglia cells (ng) that are smaller in size with small well demarcated nuclei and others with perinuclear space (nga).c) Higher magnification of the inner layers showing the inner pyramidal layer contains large pyramidal cells (P) with vesicular nuclei and basophilic cytoplasm. The acidophilic neuropil (*) contains blood vessels with narrow perivascular spaces (bv) and normal neuroglia cells (ng) and (nga). (a) Scale bar= 100 µm, x200; (b,c) Scale bar= 50 µm, x400.

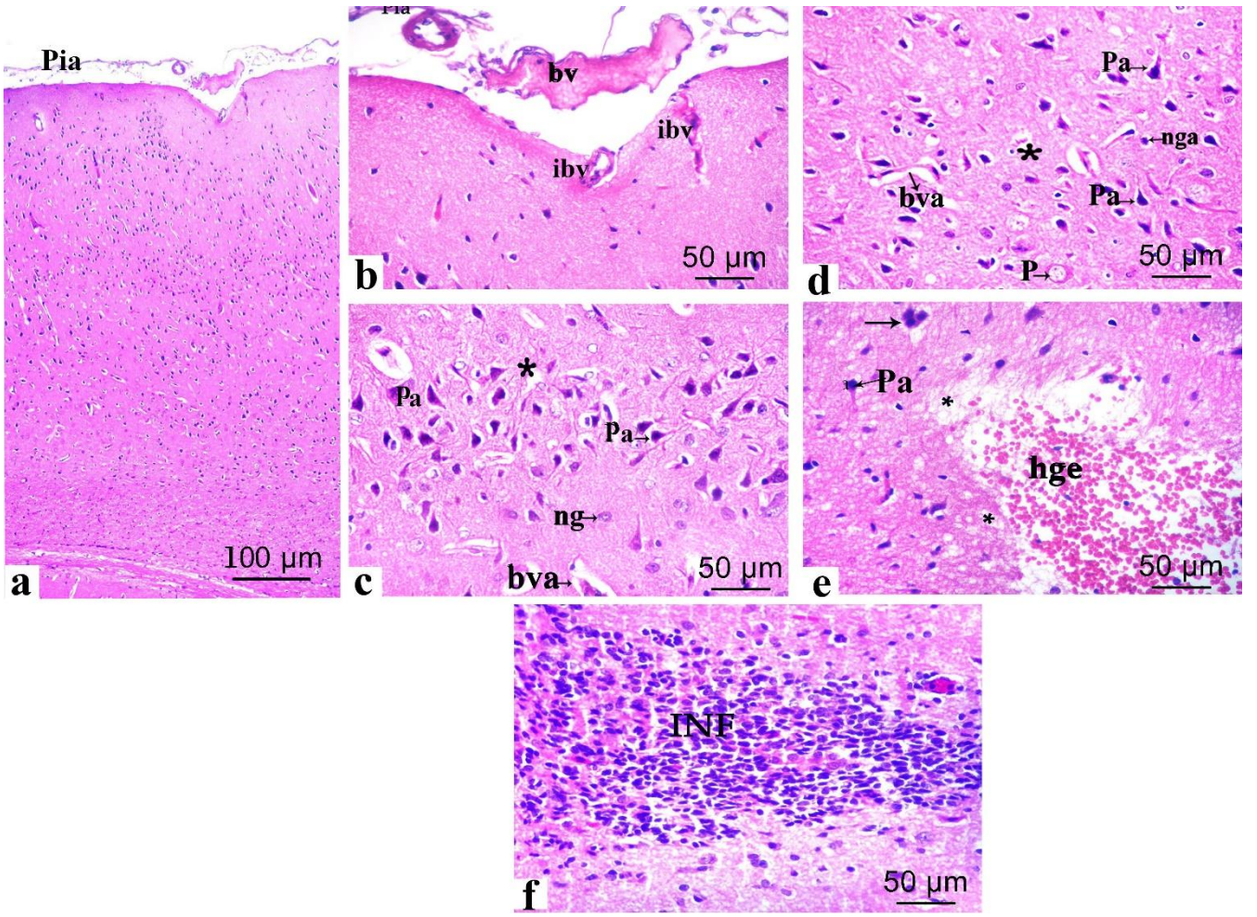

Fig. 6: photomicrographs of sections in a rat's cerebral cortex of CYP-treated group a) showing marked disorganization of the six layers of the cerebral cortex with marked separation of pia matter (pia). b) A higher magnification of the previous section showing marked separation of pia matter (pia). Congested dilated blood vessel (bv) and invasive blood vessels (ibv) are also seen. C) A higher magnification in outer layer of cerebral cortex shows most of neurons are affected most probably pyramidal cells (pa) with long process, shrunken, dark stained nuclei and surrounded by pericellular halo. Vacuolated neuropil (*) , neuroglial cells (ng) and congested dilated blood vessels (bva) surrounded by wide perivascular space can be observed. d) A higher magnification in inner layer shows affected neurons most properly pyramidal neurons (pa) have shrunken, dark stained nuclei with long process and surrounded by pericellular halo. The neuropil is vacuolated (*) and contains neuroglial cells (nga) and blood vessel (bva) that surrounded by wide perivascular space. Normal pyramidal cell (P) can be seen. e) showing area of hemorrhage (hge) and affected pyramidal neurons (pa) with dark stained nuclei. A cell contains multiple nuclei (arrow) and highly vacuolated neuropil (*) are seen. f) Other section showing marked cellular infiltration (INF). H&E (a) Scale bar= 100 µm, x200; (b, c, d. e, f) Scale bar= 50 µm, x400**.**

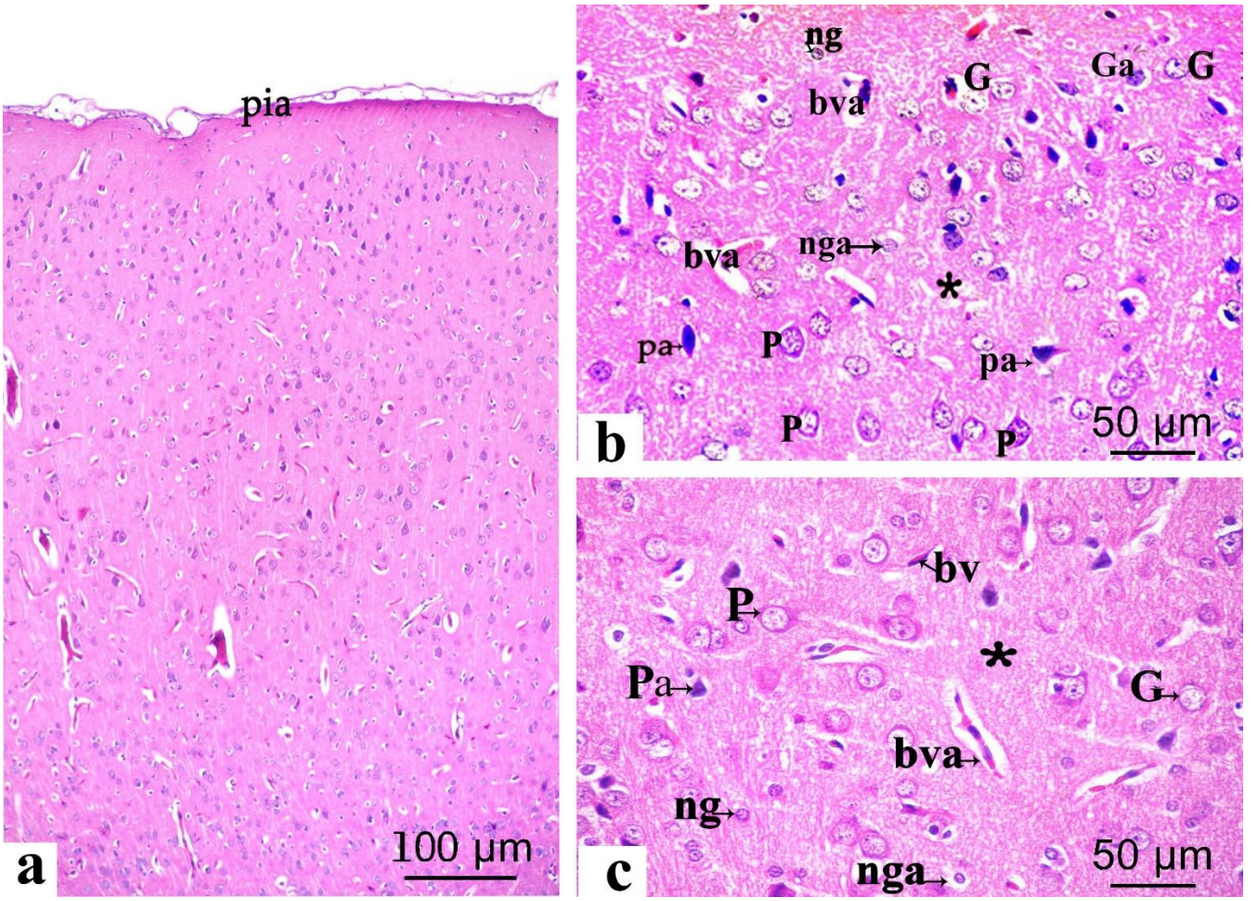

Fig. 7: photomicrographs of a section in a rat's cerebral cortex of CYP + HT-treated group a) showing mild disorganization of six layers of the cerebral cortex and little separation of pia matter (pia). b) A higher magnification of outer layers of a rat's cerebral cortex of CYP +HT-treated group showing multiple normal small conical-shaped pyramidal cells (p) and few affected pyramidal cells (pa). Most of granule cells (G) are normal with few affected granule cells (Ga). Some normal neuroglial cells (ng) and others affected ones (nga) are seen. The neuropil (*) is piof a rat's cerebral cortex of CYP + HTtreated group showing normal pyramidal neurons (P) with vesicular nuclei and basophilic cytoplasm and few affected pyramidal cells (pa) with dark stained nuclei. Few granule cells (G) with pale open face nucleus and little cytoplasm can be seen. The neuropil is less vacuolated (*) and contains blood vessel (bva) surrounded by wide perivascular space and other normal blood vessel (bv) with narrow perivacsular space. Neuroglial cells (ng) with well demarcated nuclei and others with pericellular space (nga) can be seen.

H&E (a) Scale bar= 100 µm, x200; (b,c) Scale bar= 50 µm, x400.

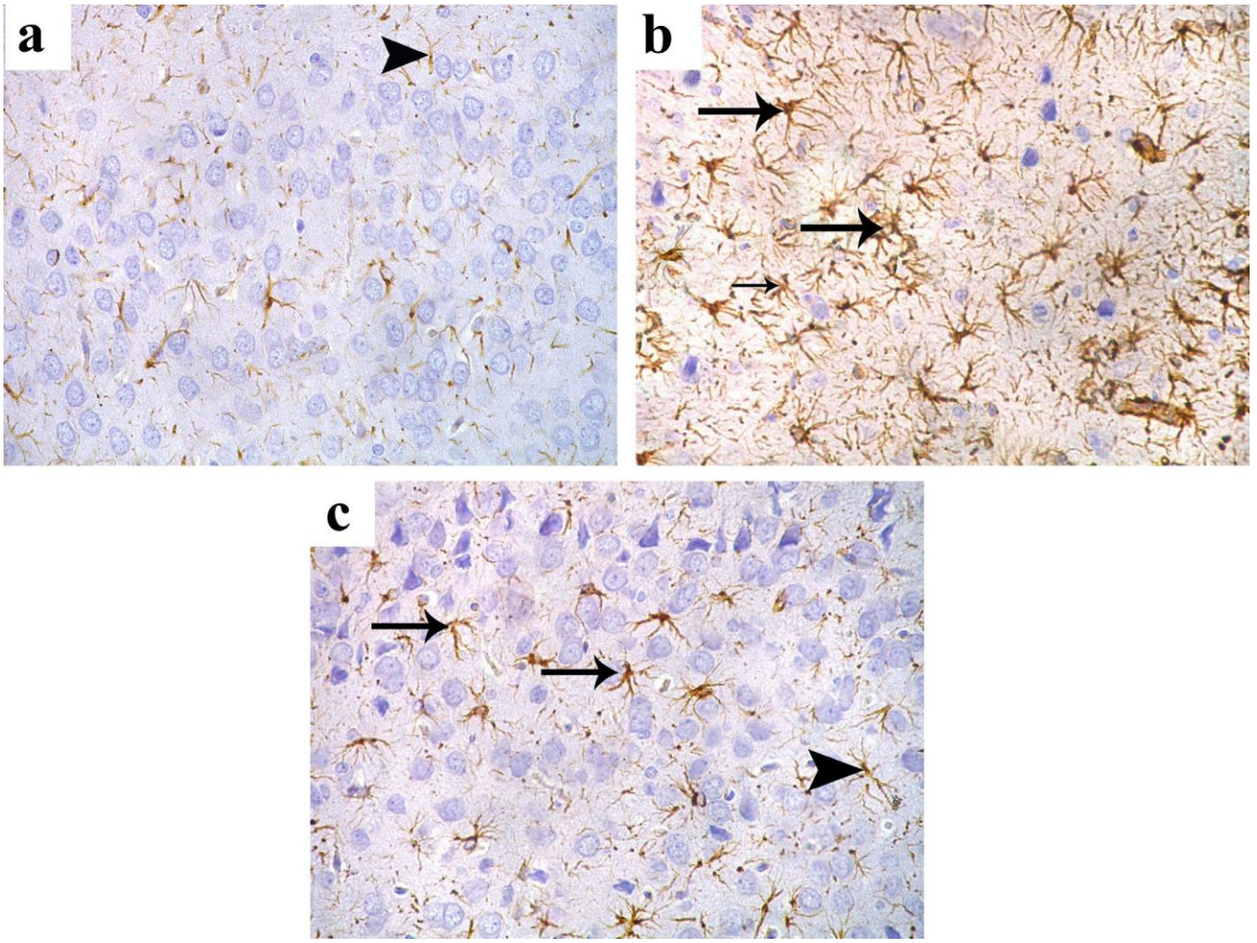

Fig. 8: photomicrographs of immunohistochemical staining of GFAP in the three different studied groups. a) Control group showing positive staining in the processes and cytoplasm of astrocyte which appear few in number and small in size with few thin short ramified processes (arrow head). b) CYP treated group showing abundant GFAP positive staining in astrocytes' processes and cytoplasm, which increase in number with multiple thick long processes (arrow). c) CYP+HT treated group showing few astrocytes, which appear large with long thick processes (arrows) and others small in size with few thin short, ramified processes (arrow head). Immuno-peroxidase technique for GFAP X 400.

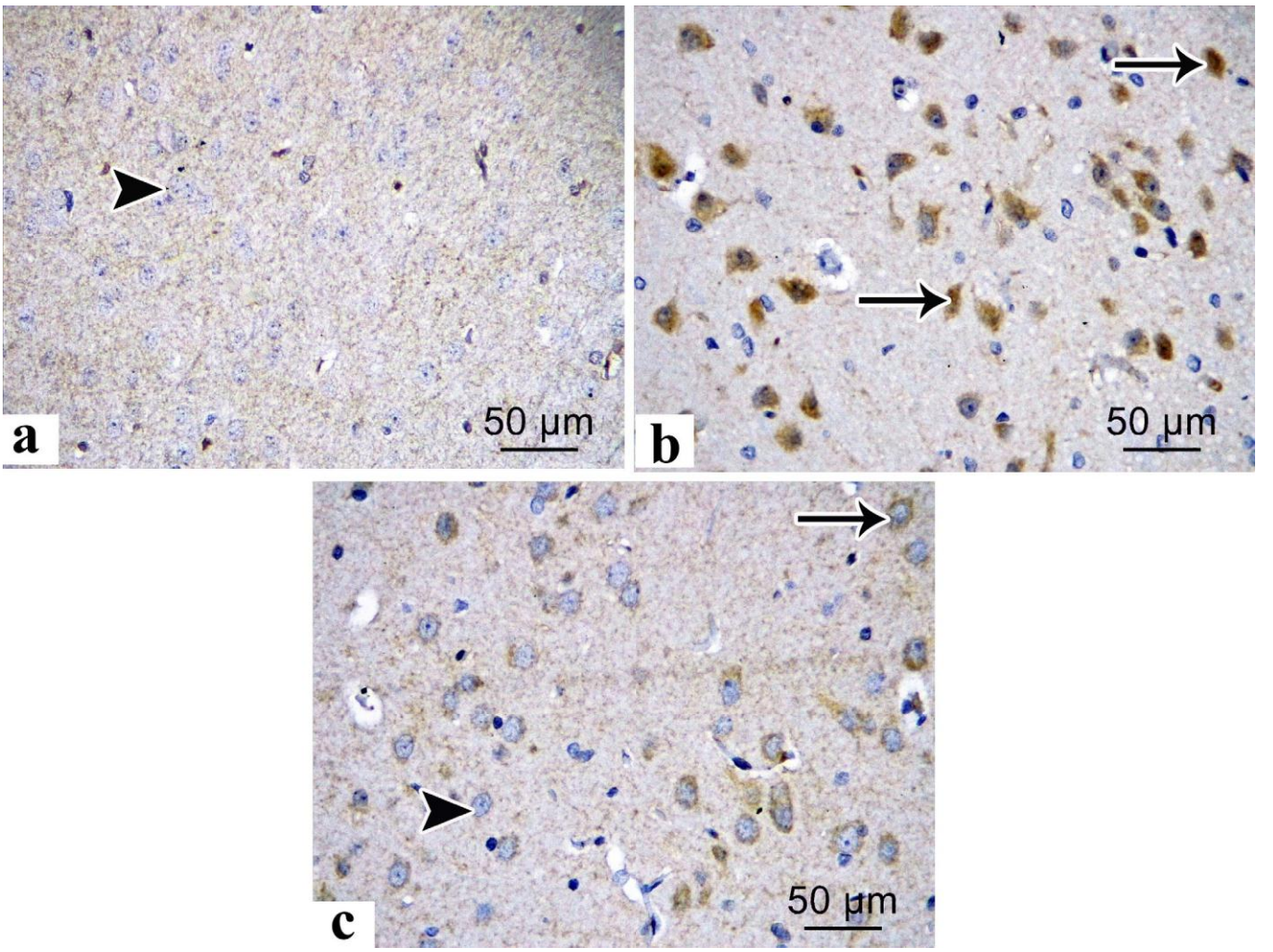

Fig. 9: photomicrographs of immunohistochemical staining of caspase-3 in the three different studied groups. a) Control group showing negative immunoreactions to caspase -3 (arrowhead). b) CYP treated group showing multiple neurons have positive immunoreactions to caspase -3 in their cytoplasm (arrow). c) CYP+HT treated group showing few neurons have positive immunoreactions to caspase -3 (arrow) and others are negative immunoreactions (arrow heads) . Immunoperioxidase technique for caspase-3, x 50 µm, x400.

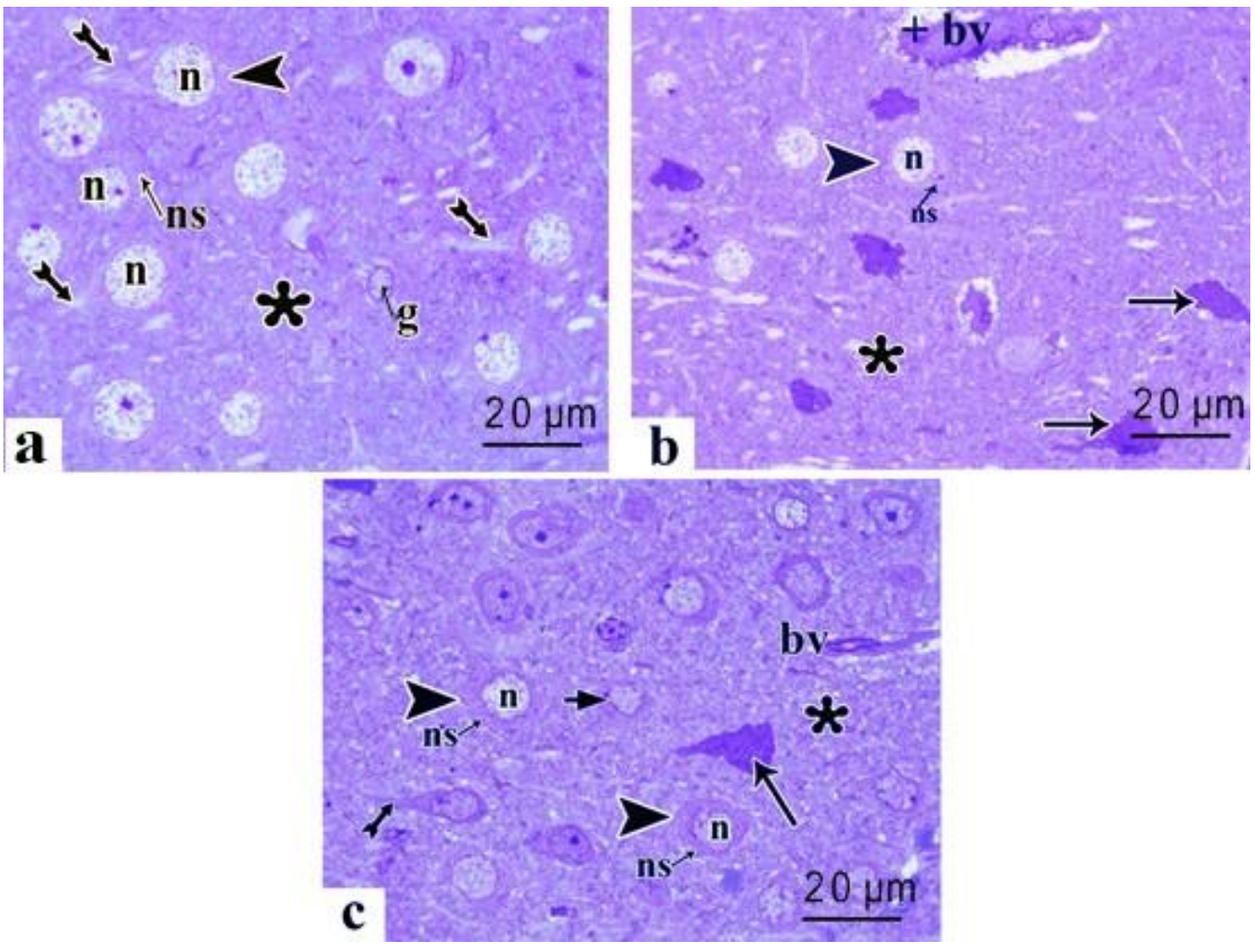

Fig. 10: photomicrographs of a semithin section in the cerebral cortex of the three studied groups a) control group showing normal neurons (arrow head) with large open-faced nuclei (n) that surrounded by pale cytoplasm containing basophilic Nissl's granules (ns). Neuronal axons can be detected (bifid arrow). Intact neuropil (*) and glial cell (g) with sharply rounded demarcated nuclei can be observed b) CYP treated group showing deeply stained cortical neurons (arrow). normal neurons (arrowhead) with large open-faced nuclei (n) surrounded by pale cytoplasm containing basophilic Nissl's granules (ns) are also noticed. Vacuolated neuropil (*) and blood vessel (bv+) with a wide perivascular area are observed. c) CYP+ HT treated group showing most neurons are normal (arrowhead) with large open-faced nuclei (n) that surrounded by pale cytoplasm containing basophilic Nissl's granules (ns). Neuronal axons can be detected (bifid arrow). Few deeply stained neurons (arrow) can be observed. Intact neuropil (*) and blood vessel (bv) with narrow perivascular space can be noticed.

Toluidine blue Scale bar= 20 µm x1000.

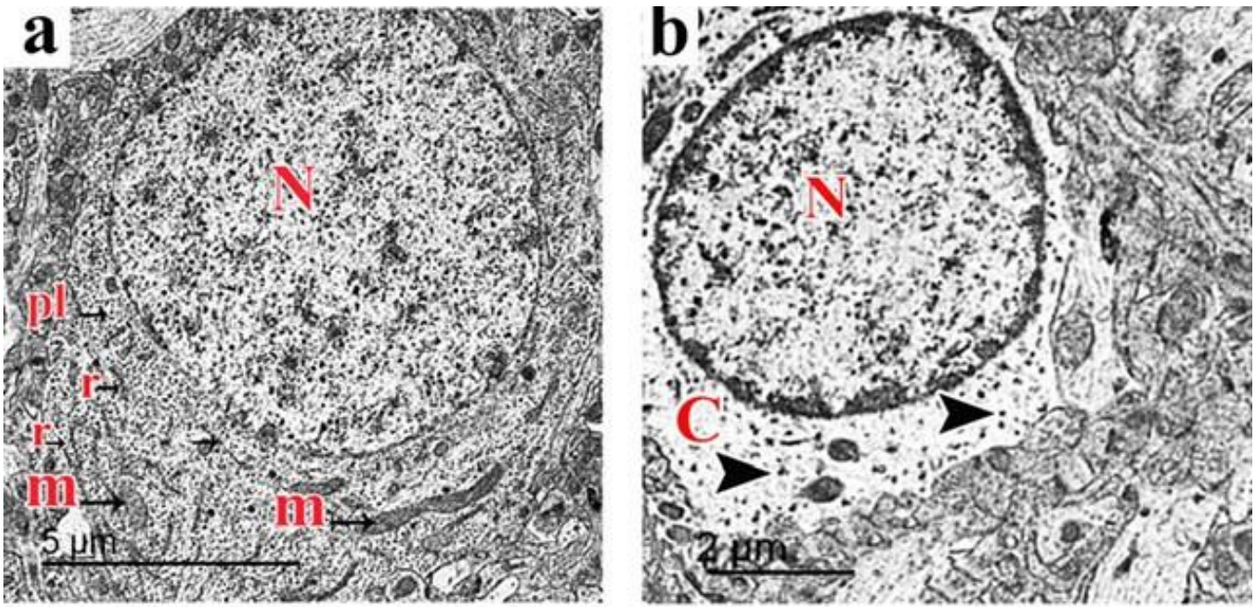

Fig. 11: Electron photomicrographs of the cerebral cortex of control group showing (a) a cortical neuron nucleus which is appeared large and euchromatic (N). The cytoplasm shows mitochondria (m), rough endoplasmic reticulum (r), and broadly diffused polysoms (pl). (b) an astrocyte (As) having a well-demarcated rounded euchromatic nucleus (N).The cytoplasm (C) is electron lucent and few dispersed glycogens are seen in it (arrow head). a) Scale bar= 5 µm, b) Scale bar= 2 µm.

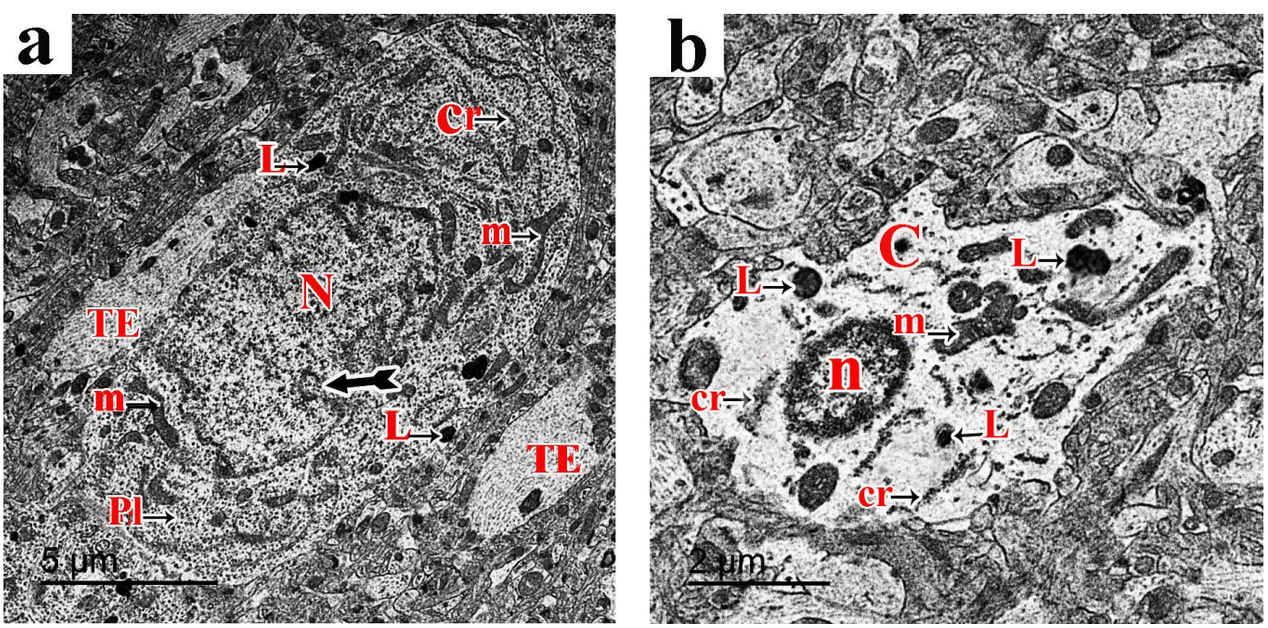

Fig. 12: Electron photomicrograph of the cerebral cortex of CYP treated group showing (a) a cortical neuron with an irregularly outlined nucleus (N) with Multiple indentations (bifid arrow) in the nuclear envelope are seen. Its cytoplasm contains dumbly shaped mitochondria (m), spread of polysomes (pl), few scattered cisterna of the dilated cisterna of rough endoplasmic reticulum (cr), and electron-dense bodies (L). Terminal end dilatation can be observed (TE). (b) an astrocyte having a rounded shrunken heterochromatic nucleus (n) and wide irregular electron lucent cytoplasm (C). Swollen mitochondria (m), dilated cisterna of rough endoplasmic reticulum (cr) and electron-dense bodies (L) are seen. a) Scale bar= 5 µm, b) Scale bar= 2 µm.

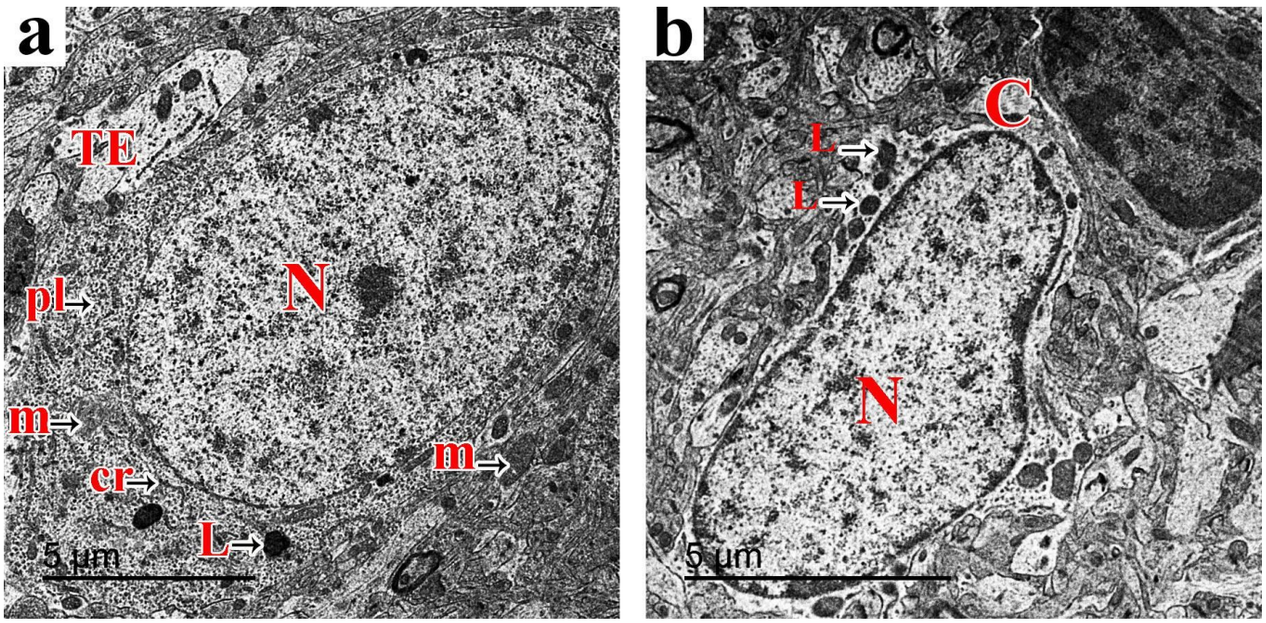

Fig. 13: Electron photomicrographs of the cerebral cortex of CYP +HT treated group showing (a) large euchromatic nucleus (N) of the cortical neuron. The cytoplasm shows mitochondria (m), rough endoplasmic reticulum (cr), electron-dense bodies (L) and broadly diffused polysomes (pl). Terminal end dilatation can be observed (TE). (b) An astrocyte having slightly irregular euchromatic nucleus (N). Narrow electron lucent cytoplasm (C) is observed. The cytoplasm shows electron-dense bodies (L) a) Scale bar= 5 µm, b) Scale bar= 5 µm.

DISCUSSION

Cypermethrin (CYP) is a synthetic pyrethroid, which is a commonly used pesticide in agricultural regions[25]. Some studies have shown that synthetic pyrethroids pesticides are toxic to the CNS and this is mainly mediated by hyper-excitation of the CNS. CYP crosses blood-brain barrier due to its lipophilic nature. It significantly impairs the development, maturation, and functioning of the blood-brain barrier. It generates neurotoxicity in CNS and motor deficits [5].

In the present study, CYP treated rats showed signs of toxicity represented by loss of weight due to decreased feeding and increased bowel movement, Irritability, itching, suspended tail, erected furs and startle response. In this group, crawling gait was developed with splaying of the hind limbs during the period of the experiment. Two rats died during the administration of CYP. These findings are inconsistence with[13] who stated that orally CYP dose at (20 mg/kg/day) resulting in moderate toxicity as decreased food intake, diarrhea, loss of body weight, ataxia, dyspnea, salivation and thick eye discharge. They reported at this dose three albino rats died.

Igho & Afoke[26] reported that oral administration of pyrethroids pesticide gave rise to suspended tail, aggressiveness, erected furs, itching, drowsiness and decrease in their feeding nature. They explained their behaviors as a result of peripheral nervous system excitation by pyrethroids pesticide. They recognized that pyrethroids penetrate the BBB causing severe neurotoxic effects on animals. Also Nair *et al*.[27] stated that oral administration of CYP orally caused salivation, startle response, seizures and abnormal movement including hind limbs as a result of increase of hind limb extensor tone. They clarified that all these signs proposed that CYP affects the nervous system and the main neurotoxic aim sites are the voltage-dependent sodium channels in the excitable membrane. The long lasting prolongation in sodium current induced by CYP, resulting in marked repetitive activity.

Findings of the current study are against with those of Cao *et al*.[28] who documented that there were non-significant signs of toxicity with the dose of (20 mg/kg). They also reported that anxiety, licking and scratching were detected and followed by convulsions, tremors, hunched posture, crawling gait with opened hind limbs, and ataxia at higher doses of (40 mg/kg and 80 mg/kg).

The hyperexcitability and neurotoxicity symptoms produced by CYP is due to decrease in GABA level. GABA is the main inhibitory neurotransmitter in the CNS which stops over shots of the nerve cells[29]. Also Kumer *et al*.[5] reported that GABA is one of the most common aims of CYP.

The results of the present study supported the previous theory; there was statistically significant decrease in mean brain GABA concentration of CYP-treated group in

comparison to control group. This result is in 29 agreements with Kumar *et al*.[5] & Manna *et al*.,[30] who stated that CYP leading to significant decrease in GABA level.

This result is against Han *et al*.[31] who stated that increased level of the GABA was detected in the cerebral cortex of mice exposed to CYP in a dose of (20 mg/kg).

Marigoudar *et al*.[32] documented that inhibition of AChE activity results in a buildup of acetylcholine (ACh). Ach produces prolonged excitatory postsynaptic potential leading to hyper-stimulation of the nerve and muscle fibers then subsequently tetany, paralysis, and eventually death. In the present study, there was a non-significant decrease in mean brain AchE concentration in CYP-treated group in comparison to control group. This result is in agreement with Sayim *et al*.[33] who stated that there was a non-significant change in AChE activity between the CYP-treated group and control group.

This results in contrary with Tayebati *et al*.[34] who stated that there was no change in level of AChE after treatment with deltamethrin. Also, Hussien *et al*.[35] reported that exposure to CYP leading to significant decrease in AChE that lead to accumulation of ACh.

Regarding to the results of weight, there was a statistically significant decrease in mean body weight and mean relative brain weight in comparison to control group in the last day of the experiment. This is in consistence with Sayim *et al*.[33] who stated that there was a significant decrease in relative brain weight of rats treated with CYP. Also, these results are in agreement with Aroonvilairat *et al*.[36] who stated that the body weights of the groups that treated with chlorpyrifos, CYP and captan, were significantly decreased.

The brain is a lipid rich organ, in which oxidation and antioxidation are maintained in a balanced state. However, brain injury produces great quantities of oxygen free radicals, which persuade oxidative stress and brain damage[37]. MDA is a main metabolite of lipid peroxidation that is brought by reactive oxygen species and therefore may designate the degree of oxidative stress present. Aouey *et al*.[38] and AlKahtane,*et al*.,[39] stated that the neurotoxic effect of CYP occur through lipid perioxidation and other oxidative damages.

The biochemical results of the current study supported the previous theory as assessment of oxidative markers revealed significant decrease in mean brain antioxidant biomarkers levels (GPx and SOD) and significant increase in MDA level of CYP-treated group in comparison to the control group. These findings are in consistence with Hussien *et al*.[35] who stated that CYP significantly reduced the GSH level and the activities of GPx, GST, SOD and CAT oxidative enzymes in the brain. Also Das *et al*.[40] reported that MDA level of erythrocytes was significantly increased in CYP treated groups.

Obinna and Kagbo[41] reported that throughout pyrethroid metabolism, ROS are created leading to oxidative stress. Additionally, Other studies were reported that after CYP administration, there were significant decreases in GPx activity. They added that significant reduction in the activity of SOD may be due to the decline in the capability of tissues to deal with extra free radical[42,43,44].

The histopathological results of the current work supported the neurotoxic effect of CYP on the cerebral cortex of the rats where hematoxylin and eosin stained sections of the cerebral cortex revealed marked histological changes in all layers of the cortex in comparison to the control group. Marked separation of pia matter and loss of normal organization of the cerebral cortex six layers were detected. Most of neurons were affected most probably apoptotic cells. Also cellular infiltration and hemorrhage were found in some areas. These findings were confirmed ulterstructurally where transmission electron microscopy showed that there were evident morphological changes in the cerebrum neurons of rats treated with CYP, of which shrunken cortical neurons with heterochromatic nucleus was observed. The nuclear envelop was irregular and characterized by the presence of indentations. The Cytoplasm was characterized by the dilated cisterna of the rER , few scattered rER, deformed mitochondria, few spread Nissl's granules, spread of polysomes (pl) , and electron-dense bodies

These findings were in consistence with those of Grewal *et al*.[13] who stated that oral administration of CYP in a dose of (20 mg/kg/day) resulted in neuronal degeneration, vacuolization and necrosis. Hasan *et al*.[45] reported that inhalation of pyrethroid created loss in organization of cortical layers. Vacuolation in cerebral cortex, inflammatory cells infiltration and degenerative neuronal changes with light-stained nuclei were detected. Muthuviveganandavel *et al*.[46] who gave CYP intradermal reported that CYP resulted in congestion and vacuolation in the brain.

GFAP is an intermediate filament protein identified to be specifically expressed in astrocytes, the glial cells that are responsible for restoring and scarring of the brain after injuries[47]. Astrocytes form the majority of the glial cell that respond rapidly to several neurodegenerative changes, leading to marked astrogliosis (48 Strużyńska *et al*., 2006).

Our results of the current work revealed that GFAP immuno-histochemical staining of CYP-treated group showed abundant GFAP positive immuno-expression of the cytoplasm and processes of astrocytes. They were actually increased in number and seemed larger with numerous thick processes in comparison to control ones.

Also the characterization of phagocytic activity as revealed by the presence of dense bodies and lysosomes in electron field.

This result is in agreement with that of Tayebati *et al*.[34] who informed that there was higher number and enlarged size of GFAP-immuno-reactive astrocytes in rats treated with deltamethrin. Also Grewal *et al*. (13) stated that oral administration of CYP in a dose of (20 mg/kg/day) resulted in obvious increase in glial cells. Krishnan *et al*.[49] articulated that, the mice group exposed to Chlorpyrifos (organophosphate pesticide) showed greater expression of GFAP in the pattern of brown coloration glial fibers in cerebellar cortex in comparison to control ones. Cheng *et al*.[50] reported that the increase in GFAP-immuno-positive astrocytes might reflect compensatory action against neuronal cell damage

In contrary, Maurya *et al*.,[3] stated that, oral treatment of rats with cypermethrin at low dose affects astrocytes, manifested by a decrease in the glial fibrillary acidic protein (GFAP) in the rat brain cortex.

Oxidative insults of cellular and nuclear biomolecules are considered as crucial triggering influences of apoptosis-related proteins[51]. Therefore, we carried out an immuno-histochemical expression analysis of caspase-3. We reported significantly upregulation of caspase-3 immune-positive cells compared to control group. Green and Reed[52] documented that Caspase-3 is a perilous initiator of apoptosis being responsible for the proteolytic cleavage of many key proteins, which damage initiates the cell death programme. These results in accordance with Abd El-Hameed & Mahmoud[53] who stated that CYP could be an apoptosis inducer and its effects are via activation of some apoptotic regulated genes in the brain and testis tissues, including p53 and Caspase-3 which were significantly up-regulated. Additionally, Zhou *et al*.,[2] reported that in mouse cortical neurons apoptosis was induced by CYP with increase in the dose and exposure time

Hydroxytyrosol (HT) is a phenolic compound that is found in olive leaf. It is one of the most powerful antioxidants in the nature. It is the only phenol that passes BBB leading to catch the free radicals in the CNS[54].

Concomitant use of HT along with the CYP led to delayed appearance of sign of hyper excitability but the rats were normal in their gait. The present results revealed a significant increase in mean values of GABA concentration comparison to CYP-treated group. This result is in agreement with Bawazir[55] who reported that olive oil resulted in significant increase in GABA in different brain areas. Also, there was increase in mean brain ACh concentration of CYP and HT-treated group in comparison to CYP-treated group but it is non-significant. This result is in agreement with Nugroho *et al*.[56] who stated that HT significantly inhibited ACh E leading to increase in ACh level.

Also co- administration of the HT along with the CYP improved the feeding, the bowel movement, the mean body weight and relative brain weight. There is no mortality in this group.

Our biochemical results supported the previous theory that the HT has antioxidant properties as there was significant increase in mean brain antioxidant biomarkers levels (GPx, and SOD) and significant decrease in MDA level of CYP and HT-treated in comparison to CYP-treated group. These finding are in consistence with Ebrahimi & Moghaddam[57] and Soni, *et al*.[58].

Alsemeh *et al*.,[59] stated that protective effect of HT can lead to significant reduction in lipid peroxidation and increase of GPx, CAT and SOD enzyme activities.

Schaffer *et al*.[9] & Elfekia[60] reported that HT and oleuropein are present in virgin olive oil which is easily absorbed by the body and have effective free-radical scavenging characters. They reported that administration of even low doses of olive oil phenols could significantly enhance the antioxidant levels in the body and decline oxidative stress in humans and animals.

Consequently, the antioxidant effect of the HT is redirected on the histological structure of the rat cerebral cortex where co-administration of the HT with the CYP produced restoration of the normal appearance of rat's cerebral neurons and re-establishment of the normal organization of the cerebral cortex layers. González *et al*.[61] stated that HT that presents in virgin olive oil had neuroprotective effects on the rat brain. They had related this neuroprotective effect mainly to the antioxidant properties of polyphenols.

Furthermore, HT, caused a significant down regulation in Caspase- 3 apoptotic marker compared to CYP treated group. This finding was in line with Alsemeh *et al*.,[59].

These results confirmed by Miao *et al*.[62] who stated that HT decreased Bcl-2-like protein 4 /Bcl-2 ratio, cleaved caspase-9, -3 and suppressed mitochondrial permeability transition pore opening and cytochrome c. Hence, HT inhibited apoptosis and reduced myocardial infarct size after ischemia reperfusion. Zheng *et al*.[63] proved that HT stimulated AMPK signaling activation leading to neuroprotection in db/ db mice and promoted neuronal survival in high glucose-induced neuroblastoma SH-SY5Y cell damage.

Additionally, GFAP immuno-histochemical expression appeared small with few short, thin processes in comparison to CYP-treated group This is in agreement with Cheng

et al.[50] who stated that the antioxidant baicalein is a neuroprotection through the inhibition effect of astrocyte markers modulation which are associated with response to injury.

Collectively these results suggest that HT and its phenolic components play a beneficial effect against targeting multiple pathological aspects induced by CYP and confirm the hypothesis that HT consumption is strongly associated with the positive effect against the neurotoxicity.

CONCLUSION

Hydrxytyrosol could successfully protect against neurotoxicity. Their fundamental mechanisms may be through suppression of oxidative stress-induced neuronal damage and apoptosis. HT may be considered as effective protective options for neurotoxicity induced by CYP exposure. It is recommended to supplement HT (olive oil phenol) as an antioxidant to protect the cerebrum from the toxic effect of CYP exposure.

ABBREVIATION

Cypermethrin (CYP), Hydroxytyrosol (HT), alondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and Gama AminoButyric Acid (GABA).

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Klaassen CD, Casarett, Doull's (2013) Toxicology The Basic Science of Poisons. 8th edition. McGraw-Hill Education P: 947-950.

2. Zhou L, Zhou M, Tan H, Xiao M, (2020) Cypermethrin-induced cortical neurons apoptosis via the Nrf2/ARE signaling pathway. Pesticide biochemistry and physiology165: 104547

3. MauryaS K, Rai A, Rai Nk, Deshpande S, Jain R, Mudiam MK., *et al*. (2012) Cypermethrin Induces Astrocyte Apoptosis by the Disruption of the Autocrine/Paracrine Mode of epidermal Growth Factor Receptor Signaling. Toxicological Sciences 125(2): 473–487.

4. Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D *et al*. (2002): Mechanisms of pyrethroid neurotoxicity. implications for cumulative risk assessment. Toxicology 171:3-59.

5. Kumar SA, Nath TM, Prakash O, Pratap S, Sharma P, Firdous S, Singh R(2014) Neurotoxic effect of cypermethrin and protective role of resveratrol in Wistar rats. International Journal of Nutrition, Pharmacology, Neurological Diseases 4(2): 104.

6. Sharma P, Firdous S, Singh R (2014) Neurotoxic effect of cypermethrin and protective role of resveratrol in Wistar rats. International Journal of Nutrition, Pharmacology, Neurological Diseases 4(2): 104.

7. De Leonardis A, Aretini A, Alfano G, Macciola V, Ranalli G(2008) Isolation of a hydroxytyrosol-rich extract from olive leaves (OleaEuropaea L.) and evaluation of its antioxidant properties and bioactivity. European Food Research and Technology 226(4): 653-659.

8. Pérez-Bonilla M, Salido S, van Beek TA, Altarejos J (2013) Radical-scavenging compounds from olive tree (Olea europaea L.) wood. Journal of agricultural and food chemistry 62(1): 144-151.

9. Schaffer S, Podstawa M, Visioli F, Bogani P, Müller WE, Eckert GP (2007) Hydroxytyrosol-rich olive mill wastewater extract protects brain cells in vitro and ex vivo. Journal of agricultural and food chemistry 55(13): 5043-5049.

10. Visioli F (2012) Olive oil phenolics: Where do we stand? Where should we go? Journal of the Science of Food and Agriculture 92(10).

11. Richards KL (2014) The most powerful natural antioxidant discovered to date-hydroxytyrosol. Available at: https://www.prohealth.com/library/the-most-powerful-natural-antioxidant-discovered-to-date-hydroxytyrosol 29641.

12. Ristagno G, Fumagalli F, Porretta-Serapiglia C, Orrù A, Cassin C, Pesaresi M, Cervo L (2012) Hydroxytyrosol attenuates peripheral neuropathy in streptozotocin-induced diabetes in rats. Journal of agricultural and food chemistry 60(23): 5859-5865.

13. Grewal KK, Sandhu GS, Kaur R, Brar RS, Sandhu HS(2010)Toxic impacts of cypermethrin on behavior and histology of certain tissues of albino rats. Toxicology international 17(2): 94.

14. IACUC (2013) Inistitutional Animal Care & Use Committe. Available at: https://research.iu.edu/doc/compliance/animal-care/iupui/iupui-iacuc-use-of-non-pharmaceutical-grade-chemicals-compounds.pdf

15. Ohkawa H, Ohishi N ,Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry 95(2): 351-358.

16. Fridovich I (1969) Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244: 6049-6055.

17. Chiu DT, Stults FH, Tappel AL(1976) Purification and properties of rat lung soluble glutathione peroxidase. Biochimica et Biophysica Acta (BBA)-Enzymology 445(3): 558-566.

18. Huo T, Chang B, Zhang Y, Chen Z, Li W, Jiang H (2012) Alteration of amino acid neurotransmitters in brain tissues of immature rats treated with realgar. J. Pharm Biomed Anal 57: 120–124.

19. Stein C, Hopfeld J, Lau H, Klein J (2015) Effects of Ginkgo biloba extract EGb 761, donepezil and their combination on central cholinergic function in aged rats. Journal of Pharmacy & Pharmaceutical Sciences 18(4): 634-646.

20. Bancroft JD, Gamble A (2008) Theory and practice of histological techniques 6th Edition, Churchill Livingstone/ Elsevier Health Sciences P: 165- 175.

21. Olga G, Irit R, Joseph W, Ofer B(2002) Role of myocardial inducible nitric oxide synthase in contractile dysfunction and β-Adrenergic hyporesponsiveness in rats with experimental volume – overload heart failure circulation. Journal of the American Heart association 105 (2): 236- 243.

22. Petrie A, Sabin C (2009) Medical Statistics at a Glance, 3rded, JohnWiley& Sons, Inc., Hoboken, New Jersey P: 165-173.

23. Balijepalli MK, Suppaiah V, Chin AM, Buru AS, Sagineedu SR, Pichika MR (2015) Acute oral toxicity studies of Swieteniamacrophylla seeds in Sprague Dawley rats. Pharmacognosy research 7(1): 38

24. Ahmad L, Khan A, Khan MZ , Hussain I (2009) Cypermethrin induced anaemia in male rabbits. Pak. Vet. J. 29:191–195.

25. Igho OE, Afoke IK (2014) A histomorphologic analysis of pyrethroid pesticide on the cerebrum and cerebellum of adult albino rats. Journal of Experimental and Clinical Anatomy 13(2): 54.

26. Nair RR, Abraham MJ, Lalithakunjamma CR, Nair ND , Aravindakshan CM(2011) A pathomorphological study of the sublethal toxicity of cypermethrin in Sprague Dawley rats. International Journal of Nutrition, Pharmacology, Neurological Diseases 1(2): 179.

27. Cao D, Chen N, Zhu C, Zhao Y, Liu L, Yang J, An L(2015) β-cypermethrin-induced acute neurotoxicity in the cerebral cortex of mice. Drug and chemical toxicology 38(1): 44-49.

28. Elsawy H, Al-Omair MA, Sedky A, Al-Otaibi L (2017) Protective effect of α-lipoic acid against α-cypermethrin-induced changes in rat cerebellum. Journal of chemical neuroanatomy 86: 52-58.

29. Manna S, Bhattacharyya D, Kumar MT., Das S (2006) Sub-chronic toxicity study of alpha-cypermethrin in rats. Iranian journal of pharmacology and therapeutics 5(2); 163-0.

30. Han Y, Cao D, Li X, Zhang R, Yu F, Ren Y, An L (2014) Attenuation of γ-aminobutyric acid (GABA) transaminase activity contributes to GABA increase in the cerebral cortex of mice exposed to β-cypermethrin. Human & experimental toxicology 33(3): 317-324.

31. Marigoudar SR, Ahmed RN, David M (2009) Cypermethrin induced: in vivo inhibition of the acetylcholinesterase activity in functionally different tissues of the freshwater teleost, Labeo rohita (Hamilton). Toxicological and Environ Chemistry 91(6): 1175-1182.

32. Sayim F, Yavasoglu NÜK, Uyanikgil Y, Aktug H, Yavasoglu A , Turgut M(2005) Neurotoxic effects of cypermethrin in Wistar rats: a haematological, biochemical and histopathological study. Journal of health science 51(3): 300-307.

33. Tayebati SK, Di Tullio MA, Ricci A, Amenta F (2009) Influence of dermal exposure to the pyrethroid insecticide deltamethrin on rat brain microanatomy and cholinergic/dopaminergic neurochemistry. Brain research 1301: 180-188

34. Hussien HM, Abdou HM, Yousef MI (2013) Cypermethrin induced damage in genomic DNA and histopathological changes in brain and haematotoxicity in rats: the protective effect of sesame oil. Brain research bulletin 92: 76-83.

35. Aroonvilairat S, Tangjarukij C, Sornprachum T, Chaisuriya P, Siwadune T, Ratanabanangkoon

K(2018) Effects of topical exposure to a mixture of chlorpyrifos, cypermethrin and captan on the hematological and immunological systems in male Wistar rats. Environmental toxicology and pharmacology 59: 53-60.

36. Nunes C, Barbosa RM, Almeida L and Laranjinha J: Nitric oxide and DOPAC induced cell death: from GSH depletion to mitochondrial energy crisis. Mol Cell Neurosci 48: 94 103, 2011.

37. Aouey B, Derbali M, Chtourou Y, Bouchard M, Khabir A, Fetoui H (2017) Pyrethroid insecticide lambda-cyhalothrin and its metabolites induce liver injury through the activation of oxidative stress and proinflammatorygene expression in rats following acute and subchronic exposure. Environmental Science and Pollution Research 24(6): 5841-5856.

38. AlKahtane, A. A., Alarifi, S., Al-Qahtani, A. A., Ali, D., Alomar, S. Y., Aleissia, M. S., & Alkahtani, S. (2018). Cytotoxicity and genotoxicity of cypermethrin in hepatocarcinoma cells: a dose- and time-dependent study. Dose-Response, 16(2), 1559325818760880.

39. Das T, Pradhan A, Paramanik A , Choudhury SM(2016) Ameliorative role of zinc on cypermethrin-induced changes in haematological parameters and oxidative stress biomarkers in rat erythrocytes;Toxicology and Environmental Health Sciences 8(3):234-246.

40. Obinna VC, Kagbo HD (2018) Effect of perinatal beta cypermethrin exposure on the biochemical profile of rat off spring. Int. J. Med. Health Res. 4(1): 7-10.

41. Gasmi S, Rouabhi R, Kebieche M, Boussekine S, Salmi A, Toualbia N and *et al*. (2017) Effects of Deltamethrin on striatum and hippocampus mitochondrial integrity and the protective role of Quercetin in rats. Environmental Science and Pollution Research 24(19): 16440-16457

42. Sharma P, Khan IA, Singh R (2018) Curcumin and quercetin ameliorated cypermethrin and deltamethrin-induced reproductive system impairment in male wistar rats by upregulating the activity of pituitary-gonadal hormones and steroidogenic enzymes. International journal of fertility & sterility 12(1): 72

43. Syed F, Awasthi KK, Chandravanshi LP, Verma R, Rajawat NK, Khanna V K , *et al*. (2018) Bifenthrin-induced neurotoxicity in rats: involvement of oxidative stress. Toxicology Research 7(1): 48-58.

44. Hasan S, Yunus SM, Maheshwari TP, Hasan N (2015) Histopathological changes in the motor cortex of rat CNS after pyrethroid based mosquito repellent inhalation–an experimental study. International Journal of Biomedical Research 6(08): 559-562.

45. Muthuviveganandavel V, Muthuraman P, Muthu S , Srikumar K(2008) A study on low dose cypermethrin induced histopathology. lipid peroxidation and marker enzyme changes in male rat. Pesticide Biochemistry and Physiology 91(1); 12-16

46. Li DR, Ishikawa T, Zhao D, Michiue T, Quan L, Zhu BL and Maeda H. (2009): Histopathological changes of the hippocampus neurons in brain injury. Histol.Histopathol., 24(9): 1113-1120

47. Strużyńska L, Dąbrowska-Bouta B, Koza K, Sulkowski G (2006) Inflammation-like glial response in lead-exposed immature rat brain. Toxicological sciences 95(1): 156-162.

48. Krishnan K, Mitra NK, Yee LS, Yang HM (2012) A comparison of neurotoxicity in cerebellum produced by dermal application of chlorpyrifos in young and adult mice. Journal of Neural Transmission 119(3): 345-352

49. Cheng Y, He G, Mu X, Zhang T, Li X, Hu J, Du G (2008) Neuroprotective effect of baicalein against MPTP neurotoxicity: behavioral, biochemical and immunohistochemical profile. Neuroscience Letters 441(1): 16-20.

50. Kilarkaje, N., Al-Hussaini, H., & Al-Bader, M. M. (2014). Diabetes-induced DNA damage and apoptosis are associated with poly (ADP ribose) polymerase 1 inhibition in the rat testis. European journal of pharmacology, 737, 29-40.

51. Green DR, Reed JC. Mitochondria and apoptosis. Science 1998; 281: 1309– 12

52. Abd El-Hameed, A. M., & Mahmoud, H. S. (2020). Cypermethrin induced apoptosis and testicular toxicity by upregulation of p53 in the brain and testis of male rats is alleviated by Sesame oil. Journal of Taibah University for Science, 14(1), 1342-1349

53. Richards KL (2014) The most powerful natural antioxidant discovered to date-hydroxytyrosol. Available at: https://www.prohealth.com/library/the-most-powerful-natural-antioxidant-discovered-to-date-hydroxytyrosol 29641.

54. Bawazir AE (2011) Chronic effect of olive oil on some neurotransmitter contents in different brain regions and physiological, histological structure of liver and kidney of male albino rats. World Journal of Neuroscience 1(03): 31.

55. Nugroho A, Choi JS, Hong JP, Park HJ (2017) Anti-acetylcholinesterase activity of the aglycones of phenolic glycosides isolated from Leonurusjaponicus. Asian Pacific Journal of Tropical Biomedicine 7(10): 849-854.

56. Ebrahimi A , Moghaddam AH(2017)The effect of olive leaf methanolic extract on hippocampal antioxidant biomarkers in an animal model of Parkinson's. Pathophysiology 5(2): 9-14.

57. Soni, M., Prakash, C., Dabur, R., & Kumar, V. (2018). Protective effect of hydroxytyrosol against oxidative stress mediated by arsenic-induced neurotoxicity in rats. Applied biochemistry and biotechnology, 186(1), 27-39.

58. Alsemeh, A. E., Samak, M. A., & Abd El-Fatah, S. S. (2020). Therapeutic prospects of hydroxytyrosol on experimentally induced diabetic testicular damage: potential interplay with AMPK expression. Cell and Tissue Research, 380(1), 173-189.

59. Elfekia A. (2009) Inhibitory action of hydroxytyrosol from glucose-induced insulin deficient and pancreas and liver toxicity in vitro;Current Trends in Biotechnology and Pharmacy 3(4): 352-36.

60. González-Correa JA, Navas MD, Lopez-Villodres JA, Trujillo M, Espartero J L and De La Cruz JP(2008) Neuroprotective effect of hydroxytyrosol and hydroxytyrosol acetate in rat brain slices subjected to hypoxia–reoxygenation. Neuroscience letters 446(2-3): 143-146.

61. Miao, J., Huang, Z., Liu, S., Li, X., Jia, P., Guo, Y.&Jia, D. (2019). Hydroxytyrosol protects against myocardial ischemia reperfusion injury by inhibiting mitochondrial permeability transition pore opening. Experimental and therapeutic medicine, 17(1), 671-678.

62. Zheng, A., Li, H., Xu, J., Cao, K., Li, H., Pu, W.,& Feng, Z. (2015). Hydroxytyrosol improves mitochondrial function and reduces oxidative stress in the brain of db/db mice: role of AMP-activated protein kinase activation. British Journal of Nutrition, 113(11), 1667-1676.

الملخص العربى

تأثير السيبرميثرين علي قشرة مخ الفأر والدور الوقائي المحتمل للهيدروكسيتيروزول

المقدمة: يعتبر السيبرمثرين مبيد حشري واسع المدى, وذو تأثير سام على الجهاز العصبي للآفات و الحشرات'حيث يتم امتصاصه بسهوله في الجهاز العصبي بعد عبوره الحاجز الدموي الدماغي في الآفات و الحشرات محدثا تسمما دماغيا لها ، ينتج عنه عجزها عن الحركة. يوجد االهيدروكسيتيروزول في أوراق و زيت الزيتون فهو مضاد للأكسدة قوي المفعول. فهو يعتبر الفينول الوحيد القادر على عبور الحاجز الدموي الدماغي و استيعاب الجذور الحرة في جميع أنحاء الجهاز العصبي المركزي.

الهدف من البحث: تهدف هذه الدراسة إلى تحديد التغيرات التى قد تحدث في القشرة المخية نتيجة التعرض للمبيد الحشري سيبرمثرين

و إمكانية الوقاية من الآثار الضارة له باستخدام الهيدروكسيتيرزول.

المواد و الطرق المستخدمة: أستخدم في هذه الدراسة ستة وثلاثون من الذكور البالغة للفئران البيضاء تم تقسيمهم إلى ٣مجموعات

المجموعة الأولى (المجموعة الضابطة): اشتملت هذه المجموعة على ١٨ فأرا تم تقسيمهم بالتساوي إلى ثلاث مجموعات فرعية:

مجموعة ١أ: لم تتلق هذه المجموعة أي علاج وأعطيت ماء وإتباع نظام غذائي متوازن.

مجموعة ١ب: يعطى كل فأر ١ ملل/كجم/اليوم من زيت الذرة بالفم لمدة ١٤ يوم.

مجموعة ١ج: يعطى كل فأر ٥٠ مجم/كجم/اليوم من الهيدروكسيتيروزول مذابا في١ ملل من الماء المقطر بالفم لمدة ١٤يوم.

المجموعة الثانية (المعالجة بالسيبرميثرين): اشتملت هذه المجموعة على ٩ فئران ، أعطى كل فأر ٢٠ مجم/كجم من السيبرميثرين مذابا في ١مل من زيت الذرةبالفم لمدة ١٤ يوم.

المجموعة الثالثة (المعالجة بالسيبرميثرين والهيدروكسيتيروزل): اشتملت هذه المجموعة على ٩ فئران ، أعطى كل فأر ٢٠ مجم/كجم من السيبرميثرين مذابا فى ١مل من زيت الذرةبالفم مع ٥٠ مجم/كجم من الهيدروكستيروزول مذابا فى١ ملل من الماء المقطر بالفم لمدة ١٤ يوم.

بعد١٤ يوم من تلقي السيبرميثرين ، تم تخدير جميع الحيوانات و استئصال المخ وتم تقسيمه نصفين. نصف للدراسات البيوكيميائية و النصف الآخر للدراسات النسيجية عن طريق صبغه الهيماتوكسيلين والايوسين والصبغه الهيستوكميائيه جى فاب.

النتائـــج: أدي التعرض للسيبرميثرين الي ظهور علامات عصبية علي الفئران كما ظهر تغيرات نسيجية للمجموعة المعالجة بالسيبرميثرين في جميع طبقات القشرة المخية مقارنة بالمجموعة الضابطة. و طبقا للدراسة الكيميائية و التحليل الإحصائي لها كشفت المجموعةالمعالجة بالسيبرميثرين انخفاض ملحوظ في القيم المتوسطة للإنزيمات المؤكسدة (GPXو (SOD زيادة في متوسط قيم MDA مقارنة بالمجموعة الضابطة. من ناحية أخرى ، كان هناك زيادة في متوسط قيم الإنزيمات المؤكسدة GPX و SOD وانخفاض ملحوظ في متوسط قيم MDA للمجموعة المعالجة بالسيبرميثرين والهيدروكسيتيروزول مقارنة بالمجموعة المعالجة بالسيبرميثرين.

الخلاصـــة: يمكن الاستنتاج أن التعرض للسيبرمثرين يسبب تأثيرات عصبية سامة و يساعد تناول الهيدروكسيتيروزول مع السيبرميثرين على تقليل هذه التأثيرات السيئة للسيبرمثرين.