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Toxicological Effects and Histopathological Alterations of Diazinon and Alpha Cypermethrin on Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Authors MAH and RMZ designed the study, wrote the protocol, performed the chemical and statistical analyses and prepared the manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: In Egypt, risk assessment of pesticides usage and pesticides misuse to protect plants and increase productivity connected with health hazards and pollution problems have been of public interest. Around the world three million acute poisoning cases from pesticide exposure annually report.
Aim of study was to investigate the histopathological alterations, biochemical and genotoxicity adverse effects of synthetic insecticides Diazinon (organophosphorus) and alpha cypermethrin (pyrethroid) which are using for agricultural and public health applications on male albino rats.
Methods: Twenty one albino rats were detached into control and two experimental groups. The experimental groups were treated with Diazinon (DIZ) and Alpha-cypermethrin (α-CYP) at sub-lethal dose (1/10 LD_{50} 36.51 and 1.4 mg/kg body weight, respectively) by oral treatment for

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consecutively 28-days. The male albino rats in control treatment were put in similar surrounding conditions and treated with 5 ml /Kg tap water. Albino rats were killed after completing exposure of 28-days, liver and brain samples were dissected out for processing and sectioning examinations.

Results: The obtained results revealed that both tested insecticides induced oxidative damage as appearance by a significant raise in malondialdehyde (MDA) indicating lipid peroxidation and deoxyribonucleic acid (DNA) damage indicating genotoxicity in the liver and brain. Besides, increase in GSH level and decline in enzymatic antioxidants (GST, CAT, SOD) activities were observed in liver. Marked increase was noticed in GSH levels and GST activity in brain whereas CAT and SOD antioxidant enzymes activity were significantly reduced. Histopathological studies in brain and liver revealed multiplied focal hepatic necrosis, hydropic degeneration of the hepatocytes and portal infiltration inflammatory cells in the liver. Necrosis of neurons, neuronophagia, focal gliosis and cellular oedema, congestion of cerebral blood vessel were noticed in the brain of exposed rats.

Conclusion: The histopathological examination of brain and liver tissues and oxidative damage biomarkers showed adverse effects of DIZ or α-CYP insecticides at the tested dose (1/10 LD_{50}).

Keywords: Diazinon; alpha-cypermethrin; albino rats; oxidative stress; DNA damage; histopathology.

1. INTRODUCTION

Pesticides are intensively used in agriculture against pests and diseases and to protect crops. However, their adverse effects are significant on other organisms as environmental pollution incredibly in developing countries [1].

Organophosphate insecticides induce hematological and biochemical changes. This group has been reported to have negative effects on different animal tissues including liver, kidney, immune system, pancreas, cardiac and vascular walls Organophosphate insecticides [2].

Diazinon organophosphorous compound was a broad-spectrum treatmeant that has an insecticidal activity [3]. In horticulture around the world diazinon has been widely used for fruits, lawns, and vegetables. It is also used in household and agricultural public health and veterinary purposes [4]. Diazinon mechanism of action relies on the irreparable inhibition of Acetyl Choline Estirase (AChE) at synaptic junctions of the peripheral and central nervous systems [5], Which led to the increase in (AChE) accumulation and hypercholinergic excitatory processes [6]. Hypercholinergic excitatory processes increase oxygen flow rate through brain and organs followed by excessive increase in consumption of ATP more than the rate of its production [7]. Such metabolic alterations led to production of reactive oxygen species [8]. Diazinon may motivate imbalance in the free radicals production/elimination processes with consequential induction of cellular damage [9].

Pyrethroid insecticides are hydrophobic more than other pesticides and indicating the site of action biological membrane. Their essential target site is neuronal sodium channels in which increase the sodium entry resulting in depolarization of the nerve membrane and block nerve conduction at high concentrations [10]. Pyrethroid insecticides toxicity to mammals has extradited much attentiveness because when animals exposed to pyrethroids, alteration in their pathological characteristics and physiological activities were induced [11]. The alpha-cypermethrin molecule contains α-cyano group in the phenoxybenzyl alcohol moiety, which seems to be responsible for production of long-lasting prolongation of sodium permeability; clinically distinguished by salivation and choreoathetosis. Decomposing of cypermethrin forms cyanides and aldehydes substances that can motivate creation of reactive oxygen species (ROS).

Extended occupational exposure for many environmental chemicals may cause oxidative stress, as a mechanism underlying the adverse effects in the biological system [12]. During ordinary metabolic functions, highly free radicals are created at the body; however, they may be inserted from the environment. Molecules are inherently unsettled as they possess one pair of electrons that turned into highly reactive. They react with cellular molecules as lipids, carbohydrates and proteins and disrupt them. As end result of this, animated cellular structures and functions are lost and eventually resulting in various pathological conditions. Continuously, ROS are formed in low concentrations as secondary product from cellular metabolism. ROS are indispensable for many physiological operations, including apoptosis, protein phosphorylation, cell discrimination, transcription

factor activation, steroidogenesis, cell immunity and cellular defense mechanism [13]. However, when ROS production increases over the normality of the body, ROS can damage cell functions as they can harm cellular proteins, DNA and lipids [14].

Some studies considered pesticides as chemical mutagens and possess genotoxic properties which lead to chromosomal alterations, DNA damage or mutations [15]. Genotoxic effects of agricultural pesticide chemicals are considered among the most earnest exposure to such pesticides for a long time of the possible side effects. If a pesticide interact with exposed organisms nuclear DNA, it may be mutagenic or lead to heritable genetic diseases adverse effects [16]. The comet assay (alkaline single-cell gel electrophoresis) is known as genotoxicity biomarker in exposed organisms to pollutants and it is associated with the earnest health sequels in the organisms [17]. Antioxidant enzymes are able to disrupt free radicals prior they attack cellular components. Antioxidants act by decrease the free radicals energy or surrender some of their electrons for its use, thereby give rise to become stable. Additionally, they may also fracture oxidizing chain reaction to decrease the impairment caused by the free radicals.

Regarding the previous mentioned, the present study was aimed to study the adverse effects of diazinon and alpha-cypermethrin, the wide spread pesticides in agriculture and public health, through determination of certain oxidative stress biomarkers, DNA damage or histopathological alterations in albino male rats.

2. MATERIALS AND METHODS

2.1 Insecticides

Diazinon *(O,O-* diethyl *O-* [6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate) and Alpha-cypermethrin:(±)-cyano (3-phenoxyphenyl) methyl 3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropanecarboxylate. Diazinon and alpha-cypermethrin formulation were supplied as an emulsifiable concentrate of (60% and 10% EC), respectively obtained from the Central Agricultural of Pesticides Laboratory, Dokki, Giza, Egypt. Acute oral LD_{50} values of the formulation for male rats are 365.12 and 14.02 mg/kg body weight (b.w) for diazinon and alpha-cypermethrin, respectively. Diazinon and alpha-cypermethrin orally administrated in a dose of 1/10 LD_{50} for both insecticides.

2.2 Animals

Experiments were completed according to the principle guidelines of animal research adopted by Ethics of Animal Use in Research Committee (EAURC), Egypt. Twenty one male albino rats, aged about 12-weeks and weighted (160±10g), were bought from animal house unit of the Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Cairo University. Male rats were kept at animal care facilities of Central Agricultural Pesticides Laboratory (CAPL). Healthy albino male rats were acclimatized for 14 days to the experimental situation before experiment starting. During this period, rats were maintained under normal haygenic situation of temperature and humidity. Rats after acclimatization were housed in the plastic cages and placed in room with 12:12-hrs light/dark cycle and natural ventilation. Throughout the experiment, rats were given clean water and food to ad libitum.

2.3 Experimental Design

Acclimatized animals were separated into three groups, seven animals each. The first served as control group and intubated tap water by stomach tube at (5mL/kg). The second, diazinon male group, was administerd diazinon at 1/10 LD_{50} at 36.51 mg/kg b.w. The third male group was orally treated with alpha-cypermethrin at 1.40 mg/kg b.w. Treated rats orally administrated the prepared insecticides water solutions by gavage at 5 ml/kg b.w. rat using stomach tube with sepherical ball tip for 28 successive days.

2.4 Tissue Preparation

Liver and brain were removed from rats by decapitation at the experiment end 28-days, under ether anesthesia and washed with selected cold saline buffer solution. Tissues were washed and immediately stored at - 80ºC. For obtaining enzymatic extracts, tissues were homogenized in ice cold 50mM sodium phosphate buffer (pH 7.4) containing 0.1mM ethylene diaminetetra acetic acids (EDTA) yielding 10% (W/V) homogenate. The homogenates were centrifuged at 12.000 rpm for 30 minutes at 4ºC. The supernatant samples were separated and used for oxidative stress biochemical markers.

2.5 Liver and Brain Oxidative Stress Biomarkers

2.5.1 Superoxide dismutase (SOD)

SOD activity in both of liver and brain was evaluated according to the procedure of Marklund and Marklund [18] depending on the pyrogallol autoxidation absorbance. Changes at 420 nm were registered at 1minute interval for 5-minutes. Pyrogallol oxidation (50%) by SOD enzyme is defined one unit (1 U) and SOD activity was uttered as U/ml.

2.5.2 Measurement of catalase (CAT) activity

CAT activity was evaluated according to the procedure of Aebi [19]. The reaction started by adding 30 mM H_2O_2 to a proper volume of homogenate in 50 mM buffer of sodium phosphate with pH 7. Then, the absorbance was followed within 3 min at 240 nm and the CAT activity was uttered as U/ml.

2.5.3 Glutathione-S-transferase activity (GST)

GST activity was evaluated according to the procedure of Habig et al. [20]. The mixture reaction comprised of CDNB, GSH at 1mM in 50 mM buffer of sodium phosphate pH 7.4 and 50µL tissue supernatant. GST activity was determined following the changes in CDNB absorbance /min at 340 nm and the GST activity was uttered as U/ml.

2.5.4 Lipid peroxidation determination

The malondialdehyde (MDA) content, as a lipid peroxidation end product, was estimated according to the method of Ohkawa et al. [21]. Mixture reaction containing 8.1% sodium dodecyl sulfate, 20% acetic acid (pH 3.3) and 0.8% thiobarbituric acid, was put in a boiling water bath for 60 minutes. The absorbance of the supernatant obtained after vigorously shaking with mixture pyridine and n-butanol (1:15, v/v) and centrifugation shaken at 3000 rpm for 10-minutes and MDA level was uttered as nmol/ml.

2.5.5 GSH determination

Reduction of glutathione content (GSH) assessed in supernatant evaluated by the procedure of Beutler, et. al. [22]. Determination of GSH is depending on the reaction of DTNB [5, 50-dithiobis-(2-nitrobenzoic acid)] with GSH forming yellow colored chromophore that absorbs the light at 412 nm. The GSH amount in the selected tissue was calculated as nmol/ml.

2.6 Liver and Brain DNA Fragmentation

Liver and brain DNA damage was measured using a single-cell gel electrophoresis (comet) assay [23]. Half gram of macerated samples in 1 ml ice-cold phosphate buffer solution (PBS) was stirred for 5min to make a suspension. After filtration, 100µl of suspension was mixed with 600µl of low-melting agarose (0.8% in PBS). 100µl of this mixture were loaded on precoated agarose slides. The coated slides were dipped in TBE buffer (0.045 M, pH 8.4), containing 2.5% SDS for 15 min. The slides were electrophoresed in the same TBE buffer without SDS at a condition of 2V/cm for 2 hr and 100 mA. The stained slides with ethidium bromide (20 µg/mL) at 4°C in humidity were observed and DNA damage were evaluated in 100 cells for each dose level using a fluorescent microscope (with excitation filter 420-490 nm [issue 510 nm]). The comets tails lengths were estimated from the middle of the nucleus to the end of the tail with 40x rise for the count and measured the size of the comet. komet 5 image analysis software which developed through kinetic imaging, Ltd. (Liverpool, UK) was used to estimate the qualitative and quantitative extent of DNA damage in the cells. In final, tail moment calculated through the same program, generally, 50 to 100 rand.

2.7 Histopathological Examination

Samples were taken from liver and brain of rats in different groups fixed in 10% formalin saline for one day and decalcification were run by formic acid then washed with tap water. After that, the dehydration was carried with sequent dilutions of alcohol (methyl, ethyl and absolute ethyl). Cleared specimens in xylene embebed in paraffin at 56ºC in hot air oven for one day. Tissue Paraffin blocks were sectioned at 4 microns thickness. Sections of tissues were collected on slides, deparaffinized and stained by eosin & hematoxylin stain and inspected by light microscopy [24].

2.8 Statistical Analysis

Analysis of data was executed by SPSS program (Version 15) and the results were uttered as mean ± S.E. Statistical differences were determined by Duncan test for multiple comparisons after ANOVA. Differences at $P \leq 0.05$ were statistically significant.

3. RESULTS

3.1 Liver Oxidative Stress Biomarkers

The alterations in Liver superoxide dismutase (SOD) activity in DIZ and α-CYP exposed rats are shown below in Table (1). The obtained results refer that there were significant decline in SOD activity to 38.9% and 18% ($P \leq 0.05$) in liver of rats treated with DIZ and α-CYP when compared by control value, respectively. The same trend was noticed in liver CAT activity where, a significant decrease in CAT activity reached 40.2% and 41.9% ($P \leq 0.05$) in both DIZ and α-CYP treatments, respectively with compared to control value. As well as, liver GST activity was reduced in both DIZ and α-CYP administered rats. GST activity significantly declined to 7.9 % and 13.4% in both DIZ and α-CYP- implemented rats compared to liver MDA levels in control group (Table 1). Significant increase was noticed in the liver MDA in groups which were administered the DIZ and α-CYP. The increase in MDA was 35.3% and 27.2% in DIZ and α-CYP groups respectively. The results also disclosed a significant accretion in liver GSH content in the DZN-treated group only when compared to control ($P \leq 0.05$). GSH level reached 10.7% in comparing with control group in DIZ exposed rats. Finally, these results indicated a significant raise in MDA, a marked increase in GSH levels and a decline in enzymatic antioxidants (GST, CAT, SOD) activities in liver of male albino rats exposed to DIZ and α-CYP.

3.2 Brain Oxidative Stress Biomarkers

Brain SOD activity of DIZ and α-CYP exposed rats was shown in Table (2). A significant decline in brain SOD activity to 9.8% in brain ($P < 0.05$) of α-CYP treated rats while, non-significant decrease in DIZ exposed rats compared to control. Results in Table (2) indicated that brain CAT activity of DIZ supplemented rats exhibited a marked reduction reached to 14.4% in comparing with control. However, CAT activity in rats supplemented with α-CYP exhibited a significant decrease. As well as, brain GST activity was significantly increased in both DIZ and α-CYP administered rats to 22.8 % and 27.8%, respectively, compared to control. Regarding to MDA levels, a significant increase was noticed with 63.2% and 51% in DIZ and α-CYP groups, respectively, comparing to the control (Table 2). The results also revealed significant increase in brain GSH content reached 7.7% and 8.9% in DIZ and α-CYP exposed rats, respectively, comparing with control. Finally, this research indicated a significant raise in MDA, a marked increasing in GSH levels and GST activity whereas a significant reduction was noticed in CAT and SOD antioxidant enzymes activity in brain of male albino rats exposed to DIZ and α-CYP.

3.3 DNA Damage

The DNA damages were measured as percentage tail DNA in brain and liver tissues of DIZ and α-CYP- treated rats compared with control group as presented in Table (3) and Fig. (1). The results indicated that DNA damage was significantly increased in brain and liver tissues of exposed male rats to both insecticides as evidenced by an increase in both tail length and moment and increase in tail DNA%, compared to control group. Finally, this research showed DNA damage indicating a genotoxicity in the liver and brain of male albino rats exposed to DIZ and α-CYP.

3.4. Liver Histopathology

Repeated oral administration of DIZ or α-CYP in a dose of 1/10 LD_{50} for both insecticides for 28-days showed apparent morphological changes in the liver tissue (Figs. 3-5). Microscopical examination liver of rat from control group showed the normal histological structure of hepatic lobules (Fig. 2). Hydropic degeneration of hepatocytes and multiple focal hepatic necrosis associated with inflammatory cells infiltration in DIZ -treated group (Fig. 3). While liver tissues in α-CYP-treated group revealed hydropic degeneration of hepatocytes (Fig. 4) and portal infiltration with inflammatory cells (Fig. 5).

3.5 Brain Histopathology

Microscopically, the changes in the histopathological architecture of the brain after 28-days of repeated oral administration of DIZ or α-CYP in a dose of 1/10 LD_{50} for both insecticides comprised in control group, no histopathological changes, (Fig. 6), necrosis of neurons, neuronophagia (Fig. 7), focal gliosis (Fig. 8) and cellular oedema (Fig. 9). Whereas, brain tissues in α-CYP-treated group revealed congestion of cerebral blood vessel (Fig. 10), cellular oedema (Figs. 11) and focal gliosis (Fig. 12).

Table 1. Oxidative stress biomarkers in the liver of male albino rats orally exposed to 1/10 LD_{50} of diazinon or alpha-cypermethrin insecticides

Treatment	SOD (U/ml)	CAT (U/ml)	GST (U/ml)	MDA (nmol/ml)	GSH(nmol/ml)
Control	5.38^{a} ±0.13	302.28^{a}±16.86	14.07^{a}±0.28	64.96^{b}±2.39	54.08^{b}±0.83
Diazinon	3.29^{c}±0.24	180.87^{b}±6.87	12.96^{b}±0.21	87.90^{a}±3.15	59.84^{a}±1.31
Alpha-cypermethrin	4.41^{b}±0.17	175.69^{b}±10.01	12.19^{b}±0.42	82.63^{a}±4.17	56.18^{b}±1.63

Data expressed as mean ±S.E. Within each column, means with different letters are significantly different (P≤ 0.05). SOD (Superoxide dismutase), CAT (Catalase), GST (Glutathione-S-transferase, MDA (Malondialdhyde), GSH (Glutathione)

Table 2. Oxidative stress biomarkers in brain of male albino orally exposed to 1/10 LD_{50} of diazinon or alpha-cypermethrin insecticides

Treatment	SOD (U/ml)	CAT (U/ml)	GST (U/ml)	MDA(nmol/ml)	GSH(nmol/ml)
Control	5.42^{a}±0.08	39.55^{a}±1.54	1.40^{b} ±0.09	10.46^{b}±0.59	51.21^{b}±0.56
Diazinon	5.25^{ab}±0.12	33.86^{c}±0.30	1.72^{a}±0.10	17.07^{a}±1.073	55.17^{a}±1.41
Alph-cypermethrin	4.89^{b}±0.18	36.75^{b}±1.03	1.79^{a}±0.06	15.79^{a}±1.53	55.76^{a}±0.85

Data expressed as mean ±S.E. Within each column, means with different letters are significantly different (P≤ 0.05). SOD (Superoxide dismutase), CAT (Catalase), GST (Glutathione-S-transferase), MDA (Malondialdhyde), GSH (Glutathione)

Table 3. DNA damage in liver and brain of male albino rats orally exposed to 1/10 LD_{50} of diazinon or alpha-cypermethrin insecticides

Treatment	Tail moment		Tail length µm		Tail DNA %	
	liver	Brain	Liver	Brain	Liver	Brain
Control	1.60^{c}±0.04	0.98^{c}±0.05	5.07^{c} ±0.19	4.23^{c}±0.07	29.16^{c}±0.92	20.13^{c}±0.47
Diazinon	6.24^{a}±0.39	2.94^{b}±0.24	11.87^{a}±0.34	8.70^{b}±0.86	46.88^{a} ±0.94	37.58^{b}±1.59
Alpha-cypermethrin	4.57^{b}±0.30	5.36^{a}±0.47	9.87^{b}±0.32	10.23^{a}±0.73	40.17^{b}±1.37	44.79^{a}±1.63

Data expressed as mean ±S.E. Within each column, means with different letters are significantly different (P≤ 0.05).

4. DISCUSSION

Since, oxidative stress has implicated in disease process and inflammatory response as a promoter of cellular pathways; it has been in the first mechanistic studies of pesticides exposure [25]. Bioactivation and metabolism of xenobiotics are a main source of increased free radical formation that implicated in cellular injury or physiological dysfunction. Pesticides exposure is one of the causes of increased oxidative stress level and it may result in altered disease susceptibility [26].

Attack polyunsaturated fatty acid in cell membrane leading to enhancement of lipid peroxidation [27]. MDA is an important biomarker of lipid peroxidation occurred in polyunsaturated fatty acids [28]. The mechanisms of the cytotoxic effects of some pesticides are linked with residues of Lipid peroxidation of polyunsaturated fatty acid that occurs in phospholipids-basic components of cell membranes [29]. The disruption in phospholipids components of cell causes a cytoplasmic and mitochondrial membrane damage that led to increase in the production of free oxygen radicals within the cells [30]. Reduced glutathione (GSH) plays a natural balance between oxidation and anti-oxidation that regulates the vital functions of cells such as the synthesis and repair of DNA, the synthesis and activation of protein maintain the main thiol status of protein [31]. GSH and GSH-related enzymatic systems in cells drive benefit physiological roles in detoxification. GSH is a nucleophile that can interact with electrophilic species rendering the electrophilic molecules more solubility and unable to interact with cellular components [32]. Reduction in GSH levels may reduce the cellular power for destroying free radicals and reactive oxygen species. Depletion of glutathione may be linked with induction the

activity of glutathione-S-transferase (GST), where GST-mediated conjunction considered an important mechanism for detoxifying lipid peroxidation products [33].

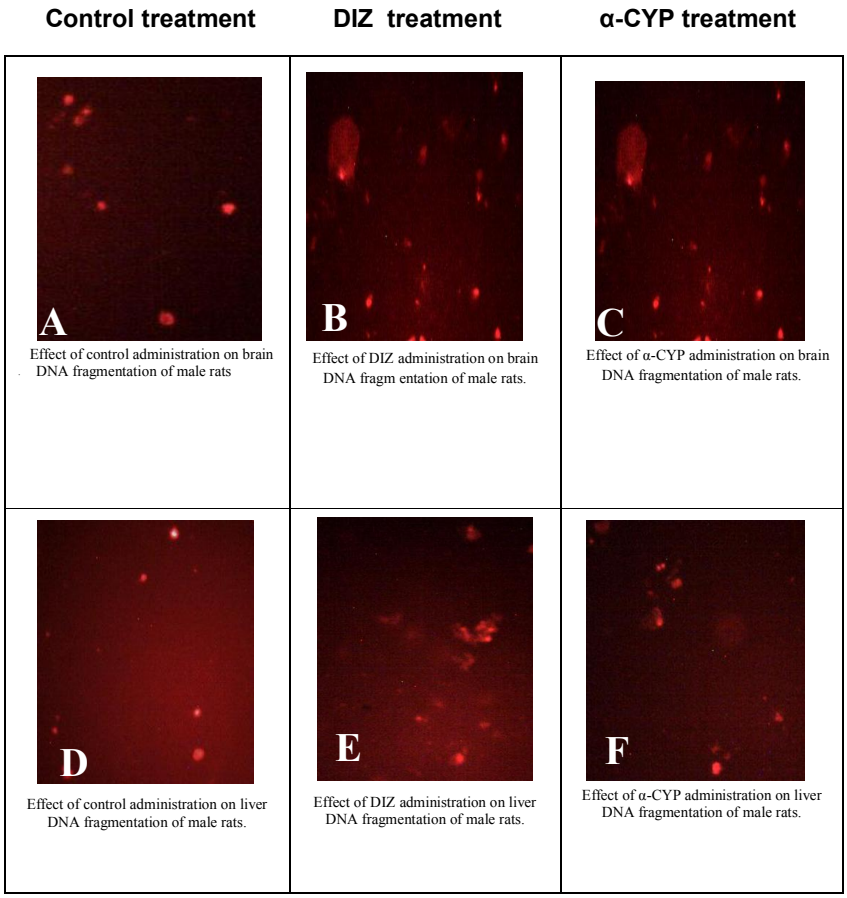

Fig. 1. Effect of DIZ and α-CYP administration on brain DNA fragmentation of male rats. (A) Control, (B) DIZ, (C) α-CYP. and effect of DIZ and α-CYP administration on liver DNA fragmentation of male rats. (D) control, (E) DIZ, (F) α-CYP.

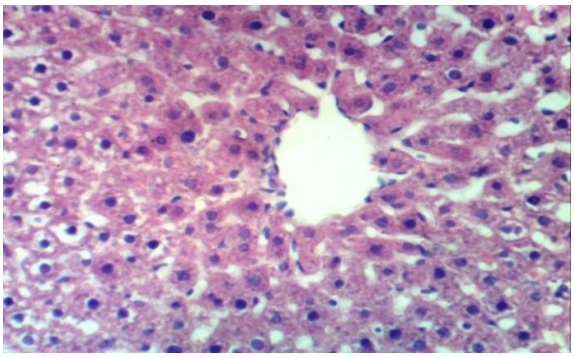

Fig. 2. Microscopical examination liver of rat from control group showed the normal histological structure of hepatic lobules.

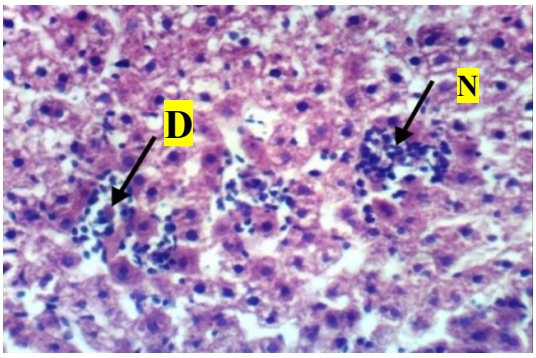

Fig. 3. Microscopical examination of liver of rat from DIZ group revealed D= hydropic degeneration of hepatocytes and multiple focal N=hepatic necrosis associated with inflammatory cells infiltration.

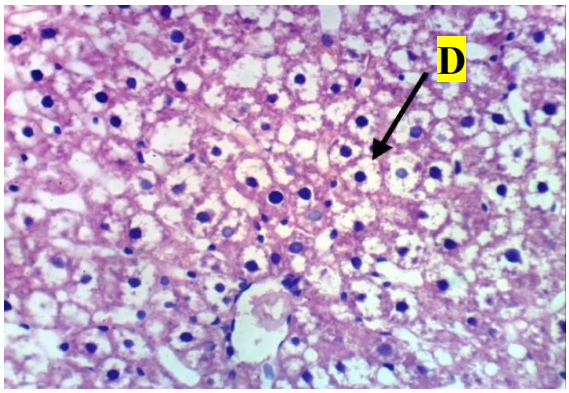

Fig. 4. Microscopical examination in examined liver of rats from α-CYP group revealed D=hydropic degeneration of hepatocytes.

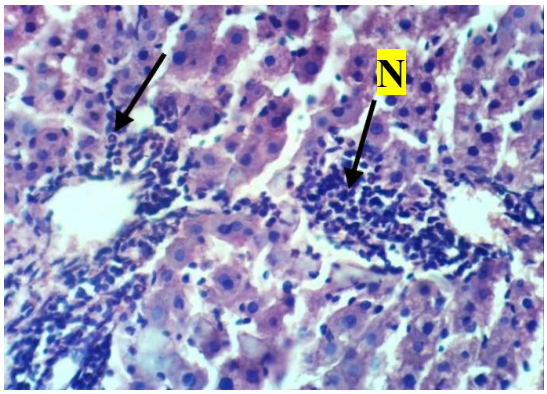

Fig. 5. Microscopical examination in liver of rats from α-CYP group revealed N= portal infiltration with inflammatory cells.

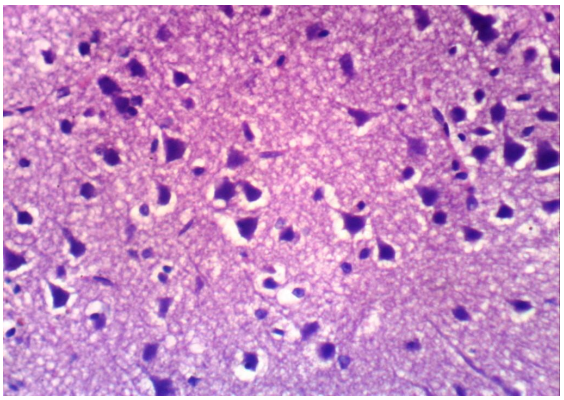

Fig. 6. Microscopical examination in brain section of control group revealed no histopathological changes.

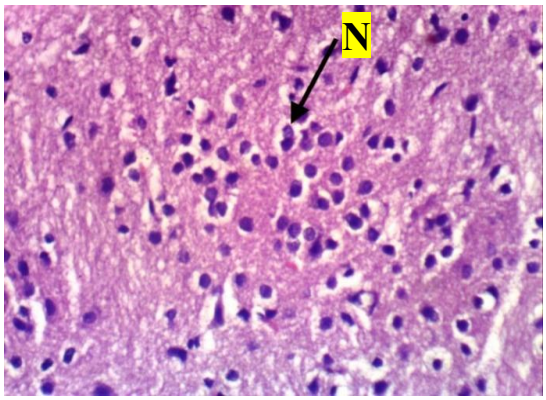

Fig. 7. Microscopicall examination in brain section of rat from group DIZ has a N=necrosis of neurons and neuronophagia.

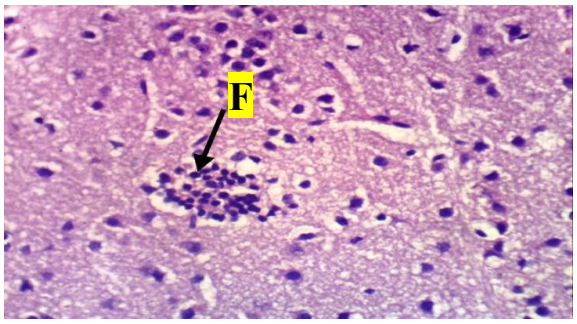

Fig. 8. Microscopicall examination in brain section of rat from group DIZ has F = Focal gliosis.

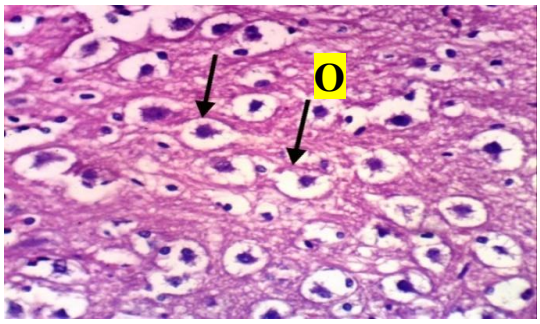

Fig. 9. Microscopicall examination in brain section of rat from group DIZ has O =cellular oedema.

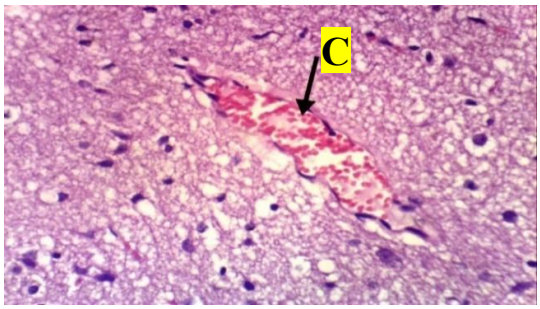

Fig. 10. Microscopicall examination in brain section of rat from group α-CYP revealed C=congestion of cerebral blood vessel.

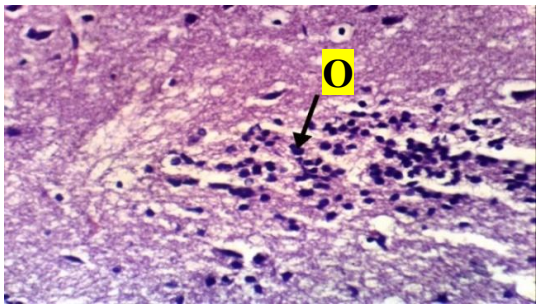

Fig. 11. Microscopicall examination in brain section of rat from group α-CYP O =cellular oedema.

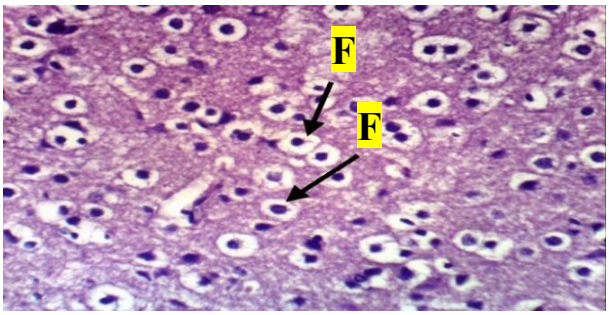

Fig. 12. Microscopicall examination in brain section of rat from α-CYP showed F = Focal gliosis.

Organophosphates caused a significant raise in MDA level and a decrease in antioxidants (GSH) content [34]. Lipid peroxidation and reduced total antioxidant capacity in rat liver of diazinon exposed rats were noticed in previous studies of [35,36] which had number of adverse biological effects by interfering with normal cell homeostasis in several ways including blocking protein metabolism [37] and interference in GSH pathways [38]. The moderate oxidative stress may increase glutathione synthesis, while the sharp oxidative stress may cause the oxidation of reduced glutathione to the oxidized form and the lowering of the antioxidant enzyme activity [39].

SOD destroy the free radical superoxide anion into hydrogen peroxide and molecular oxygen and prevents formation of hydroxyl radicals and plays a fundamental role in the cellular antioxidant mechanism [40].The decrease in SOD activity may be due to a decrease in the synthesis of SOD proteins or inactivation of enzyme proteins when over production of free radicals occurred [41]. Also, SOD activity reduction reflects oxidative stress [42]. Superoxide dismutase activity is modulated by both tissue oxygenation and generation of ROS [43]. CAT is implicated in an assortment of biochemical functions; breakdown and removal of high levels of H_2O_2 [44]. The reduction in CAT activity in the tested pesticides treatments may be attached to the decrease in SOD activity. The reduction in SOD activity reduces H_2O_2; the substrate for CAT. The lack in substrate of CAT gives rise to reduction in CAT activity. Also, accumulation of O_2^- had been shown to inhibit CAT activity [45]. O_2^- oxidized the ferrous state of CAT and led to CAT inactivation [46]. The reduction of cellular antioxidant defense mechanisms reflected the inability of tissue to scavenge excess ROS, which could be assisted as one of the reasons of ROS increase [47].

Any DNA damage such as strand breaks may be linked with cell integrity disruption consequently cell toxicity or cell death at the end [48]. DNA lesions with physiological relevance to neural cells are single strand DNA breaks, which engender from the decadence of the sugar phosphate backbone of DNA following oxidative attack by ROS [49]. Strand breaks are expressed in DNA during sugar fragmentation events and when excision repair enzymes remove damaged bases occurred [50]. Furthermore, ROS may

indirectly damage DNA via the production of oxidized lipid and protein byproducts that form adducts [51].

Pyrethroids are lipophilic insecticides that affect lipid components in lipid packing of cell membrane and lead to disturbances in cell membrane [26]. Cypermethrin hydrolysed *via* hydrolytic ester cleavage and metabolized by the CYP-450 enzymes leading to ROS [52]. This over production in ROS mediated lipid oxidation and Ca^{++} release from storage in endoplasmic reticulum and consequently caused cytotoxicity and genotoxicity in vertebrates during exposure [53]. LPO may be enhanced by free radicals created by pyrethroids and this may be the mechanism of pesticides toxicity [54].

DNA fragmentation noticed in the present research is the normal consequence of oxidative stress that was demonstrated through elevation in LPO, reduction in antioxidant enzymes and glutathione content in rat liver. This was also consistent with previous studies, where DNA fragmentation was induced by lambda cyhalothrine in rat lymphocytes [55] and by cypermethrin in rat brain [56].

Pathological examination of liver in diazinon and alpha cypermethrin exposed rats revealed hydropic degeneration of hepatocytes and multiple focal hepatic necrosis associated with inflammatory cells and portal infiltration. Inflammatory cells act as a defense mechanism to toxic materials. Similar results were observed by El-Shenawy [57] who found that mice intoxicated with diazinon (DIZ) resulted in necrosis and hydropic degeneration in the liver.

The pathological changes in brain of diazinon and alph-cypermethrin intoxicated rat revealed necrosis of neurons, neuronophagia, focal gliosis and cellular oedema and congestion of cerebral blood vessel. Microglial cells have receptors that allow them to sense damaged tissue and to recognize viruses, environmental and endogenous toxins and other pathogens. Such recognition leads to up regulate of microglial cells (Activated microglial encircle degenerating neurons (neuronophagia)). Gliosis is a nonspecific reactive alteration of glial cells in response to damage the central nervous system. In most cases, gliosis comprised the proliferation or hypertrophy of many various types of glial cells, inclusive microglia, oligodendrocytes and astrocytes.

The observed changes in the overall histoarchitecture of liver and brain organs in response to DIZ and CYP could be due to their toxic effects primarily by the generation of ROS causing damage different membrane constituents of the cell [58] or may be attributed to liver damage resulting in a reduction in antioxidant defenses in the liver [59].

5. CONCLUSION AND RECOMMENDA-TIONS

The present study showed the adverse effects of DIZ and α-CYP on male albino rats as evidenced by a significant rise in malondialdehyde (MDA), DNA damage, amarked alterations in antioxidant biomarkers (SOD, CAT, GST, GSH) and Histopathological changes in liver and brain of exposed rats. So the present study recommended to reduction of humans exposure to diazinon (DIZ) and Alpha-cypermethrin (α-CYP) pesticides.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Experiments were carried out in compliance with the guidelines of the ethical principles in animal research adopted by Ethics of Animal Use in Research Committee (EAURC), Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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