Original Research Article

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

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Abstract:

Background: In Egypt, risk assessment of usage of pesticides and pesticides misuse to protect plants and increase productivity is connected with serious problems of pollution and health hazards have been of public interest. Around the world three million acute poisoning case from pesticide exposure annually report. **The aim** of study was to investigate the histopathological alterations, biochemical and genotoxicity adverse effects of synthetic pesticides organophosphorus Diazinon (DIZ) and pyrethroid alpha cypermethrin (α-CYP) which are using for agricultural and public health applications on male albino rats.
Methods: Total of twenty one albino rats were divided into three groups, control and two experimental groups. The experimental groups was were treated with Diazinon and Alpha-cypermethrin at sub-lethal dose (1/10 LD_{50} 36.51and 1.4 mg/kg b.w., respectively) orally for 28 consecutive days. The male Albino rats in control group were put in similar surroundings and treated with 5 ml /Kg tap water. The 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 the two tested insecticides induced oxidative damage as manifested by a significant increase in malondialdehyde (MDA) and deoxyribonuclic acid (DNA) damage indicating lipid peroxidation and genotoxicity in the liver and brain,. In the contrary a marked reduction in GSH levels in liver and brain was observed. As well as, enzymatic antioxidants (GST, CAT, SOD) in both organs. Histopathological studies in liver and brain revealed hydropic degeneration of hepatocytes and multiple focal hepatic necrosis associated with inflammatory cells infiltration 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 show the adverse effects of (DIZ) and (α-CYP) pesticides at the tested dose for insecticides.

Keywords: Diazinon, alpha cypermethrin, albino rats, oxidative stress, DNA damage, Histopathology.

Introduction

Pesticides are used in intensively in agriculture to protect crops against diseases and other. However their adverse impacts on non-target organisms are

significant as well as environmental pollution and health hazards, particularly in developing countries (Assayed *et. al.,* 2010).

Organophosphate insecticides induce hematological and biochemical changes this group have been reported that, as negatively affect on different animal tissues including liver, kidney, immune system, pancreas, cardiac and vascular walls. Organophosphate insecticides (Sargazi *et. al.*, 2014)

Diazinon is a synthetic organophosphorous compound with a broad-spectrum insecticidal activity (Cakici and Akat, 2013). It has been widely used all over the world in agriculture and horticulture for lawns, fruits, vegetables and food products. It is also used in household and agricultural public health and veterinary purposes (El-Mazoudy *et. al.,* 2011). The mechanism of action of diazinon relies on the irreversible inhibition of acetylcholinesterase (AChE) at synaptic junctions of the central and peripheral nervous systems (Agrawal and Sharma, 2010), Which led to the increase in acetylcholine (ACh) accumulation and hypercholinergic excitatory processes (Adler *et. al.*, 1992). 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 (Rai *et. al.,* 2011**).** Such metabolic alterations led to production of reactive oxygen species (ROS) (Gupta *et. al.,* 2007). Diazinon may induce imbalance in the free radicals production/elimination processes with consequent induction of cellular damage **(**Gokcimen *et. al.,* 2007**).**

On the other hand, pyrethroids are more hydrophobic than other pesticides indicating that the site of action is biological membrane. Their main target site is neuronal sodium channels in which increases in sodium entry resulting in depolarization of the nerve membrane and block nerve conduction at high concentrations (Ahmet *et. al.,* 2005). Pyrethroid insecticides toxicity to mammals has received much attention because when animals exposed to these pyrethroids,

changes in their physiological activities and pathological features were induced (Saxena and Saxena, 2010). 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 characterized by choreoathetosis and salivation. Decomposing of cypermethrin forms cyanides and aldehydes substances that can induce production 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 (Abdollahi *et. al.,* 2003). During normal metabolic functions, highly reactive species called free radicals are generated in the body; however, they may also be introduced from the environment. These molecules are inherently unstable as they possess lone pair of electrons and hence become highly reactive. They react with cellular molecules such as proteins, lipids and carbohydrates, and disrupt them. As a result of this, vital cellular structures and functions are lost and ultimately resulting in various pathological conditions. Reactive oxygen species (ROS) are formed continuously in low concentrations as by-products of cellular metabolism. ROS are essential for several physiological processes, including protein phosphorylation, transcription factor activation, cell differentiation, apoptosis, steroidogenesis, cell immunity and cellular defense mechanism (Miller *et. al.*, 1993). However, when ROS production increases over the normality of the body, ROS can damage cell functions as they can harm cellular lipids, proteins and DNA (Sugino, 2006).

Pesticides have been considered chemical mutagens and some studies have shown that it possess genotoxic properties leading to mutations, chromosomal alterations or DNA damage (Bolognesi, 2003). Genotoxic effects of agricultural pesticides are considered among the most serious of the possible side effects. If a

pesticide reacts with nuclear DNA, it may be mutagenic to the exposed organisms. The exposure to such chemicals for a long period may lead to adverse effects including heritable genetic diseases (Patel *et. al*., 2007). The alkaline single-cell gel electrophoresis (comet assay) is known biomarker of genotoxicity in organisms exposed to environmental pollutants and it is associated with the serious health consequences in the organisms (Pavlica *et al.,* 2001). Antioxidant enzymes are capable of deactivating free radicals before they attack cellular components. Antioxidants act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt oxidizing chain reaction to minimize the damage caused by 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 oxidative stress biomarkers, DNA damage and histopathological alterations in male albino 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. By 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 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 carried out in compliance with the guidelines of the Ethical Principles in Animal Research adopted by Ethics of animal use in research committee (EAURC), Vet. Med. College, Cairo University, Egypt. Twenty one male albino rats aged about 12-weeks and weighted (160±10g) were obtained from the breeding unit of the Toxicology and Forensic Medicine Department, Faculty of Veterinary Medicine, Cairo University. Animals were maintained at the animal care facilities of Central Agricultural Pesticides Laboratory (CAPL). They were clinically healthy and were acclimatized to the experimental conditions for two weeks before start of the experiment. During this period, they were kept under normal environmental conditions of temperature and humidity. the rats were housed in plastic cages and placed in quiet room with natural ventilation and 12:12-hrs light–dark cycle. Clean food and water were given to rats ad libitum throughout the experimental period. Commercial standard diet and water were continuously and regularly supplied ad libitum throughout the experimental period

2.3. Experimental Design:

After acclimation period, animals were divided into three groups with 7-animals each. The first group was used as control; hence these animals were orally given tap water (5mL/kg). The second male group was orally treated with 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 solutions by gavage at 5 ml/kg b.w. rat. Using stomach tube with sepherical ball tip for 28 succesive days.

2.4. Tissue preparation:

Liver and brain were removed from rats by decapitation at the end of experiment at 28-days under ether anesthesia and washed with cold saline buffer

solution selected. Washed tissues were immediately stored at -80ºC. For obtaining enzymatic extracts tissues were homogenized in ice cold 50mM sodium phosphate buffer (pH7.4) containing 0.1mM ethylene diaminetetra acetic acids (EDTA) yielding 10% (W/V) homogenate. The homogenates were centrifuged at 12.000 rpm for 30-min at 4ºC. The supernatant samples were separated and used for biochemical markers of oxidative stress.

2.5. Liver and Brain Oxidative stress biomarkers:

2.5.1. Superoxide dismutase (SOD**):**

SOD activity in liver and brain was measured according to the method of (Marklund and Marklund 1994) depending on the autoxidation of pyrogallol. Changes in the absorbance at 420-nm were recorded at 1-min interval for 5-min. One Unit (1 U) of enzyme activity is defined as the amount of enzyme required to inhibit the rate of pyrogallol oxidation by 50%. The SOD activity was expressed as U/ml.

2.5.2. Measurement of Catalase (CAT**) activity:**

CAT activity was measured by the method of (Aebi, 1984). The reaction started by adding 30 mM H_2O_2 to an appropriate volume of homogenate in 50 mM sodium phosphate buffer with pH,7. Then, the absorbance was read at a wavelength of 240 nm within 3-min. The CAT activity was expressed as U/ml.

2.5.3. Glutathione-S-transferase activity (GST):

GST activity assayed according to the method of (Habig *et. al*., 1973). The final reaction mixture contained 1mM CDNB, 1mM GSH in 50 mM sodium phosphate buffer pH 7.4 and the reaction was initiated by the addition of 50µL

sample tissue. GST activity were determined following the changes in the absorbance of CDNB/min at 340nm The GST activity was expressed as U/ml.

2.5.4. Lipid peroxidation determination:

The malondialdhyde (MDA) content as a lipid peroxidation end product of the homogenates was determined according to the method of (Ohkawa *et. al*., 1979). The reaction mixture, containing 8.1% sodium (SDS) dodecyl sulfate, 20% acetic acid (pH 3.3) and 0.8% thiobarbituric acid, was placed in a boiling water bath for 60 min. After cooling, an *n*-butanol and pyridine mixture (15:1, v/v) was added and then shaken vigorously and centrifuged at 3000 rpm for 10-min. The absorbance of the supernatant was measured spectrophotometrically at 532 nm at room temperature. MDA level was expressed as nmol/ml

2.5.5. GSH determination:

Reduced glutathione content (GSH) estimation of supernatant was performed by the method of (Beutler *et. al*.,. 1963). Determination of GSH is based on the reaction of DTNB [5, 50-dithiobis-(2-nitrobenzoic acid)] with GSH yielding a yellow colored chromophore with a maximum absorbance at 412nm. The amount of GSH present 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 (Singh, *et. al.,* 1988), 0.5g of crushed samples were transferred to 1 ml ice-cold phosphate buffer solution (PBS). This suspension was stirred for 5min and filtered. Cell suspension af (100µl) was mixed with 600µl of low-melting agarose (0.8% in PBS). 100µl of this mixture were spread on agarose-precoated slides. The coated slides were immersed in lyses buffer (TBE (0.045M), pH 8.4, which containing 2.5% SDS) for 15-min. The slides were placed in electrophoresis chamber containing the same TBE buffer without SDS. The electrophoresis conditions were 2V/cm for 2-hr and 100 m A. The slides were

stained with ethidium bromide (20µg/mL) at 4°C and observed in humid samples. The DNA fragment migration patterns of 100 cells for each dose level were evaluated using a fluorescence microscope (with excitation filter 420-490 nm [issue 510nm]). The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40x increase for the count and measure the size of the comet. For visualization of DNA damage, observations are made of EtBr-strained DNA using a 40x objective on a fluorescent microscope. Although any image analysis system may be suitable for the quantitation of SCGE data, we used komet 5 image analysis software developed by kinetic imaging, Ltd. (Liverpool, UK) linked to a CCD camera. To assess the quantitative and qualitative extent of DNA damage in the cells the length of DNA migration and the percentage of migrated DNA were measured. Finally, the program calculates tail moment. Generally, 50 to 100 rand.

2.7. Histopathological Examination:

Samples were taken from liver and brain of rats in different experimental groups and fixed in 10% formalin saline for twenty four hours and decalcification was occurred on formic acid. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin wax tissue blocks were prepared for sectioning at 4 microns thickness. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin & eosin stain and examined by light microscopy (Banchroft *et. al*., 1996).

2.8. Statistical analysis:

Analysis of data was performed by using SPSS (Version 15) and the results are expressed as M±S.E. Statistical differences were determined by Duncan test for multiple comparisons after ANOVA. $P<0.05$ was considered statistically significant.

3. Results

3.1. Liver Oxidative Stress Biomarkers.

The alterations in Liver superoxide dismutase (SOD) activity of DIZ and α-CYP -exposed rats are shown in Table (1). The obtained results indicated that there were a significant decline in SOD activity to 61.2% and 82% of control values ($P < 0.05$) in liver of rats treated with DIZ and α-CYP, respectively. The same trend was observed in liver CAT activity where, a significant decrease in CAT activity reached 59.8% and 58.1% ($P < 0.05$) in both DIZ and α-CYP treatments, respectively with respect to control values. As well as, liver GST activity was reduced in both DIZ and α-CYP administered rats. GST activity significantly declined to 92.1 % and 86.6% in both DIZ and α-CYP- implemented rats. With respect to liver MDA levels compared to the 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 135.3% and 127.2% in DIZ and α-CYP groups respectively. The results also revealed a significant increase in liver GSH content in the DZN-treated group only when compared with the control group ($P<0.05$).GSH level reached 110.6% in comparing with control group in DIZ exposed rats. In the conclusion,the present study showed a significant increase in malondialdehyde (MDA), a marked increase in GSH levels and a decline in enzymatic antioxidants (GST, CAT, SOD) activity in liver of male albino rats exposed to DIZ and α-CYP.

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	CAT	GST	MDA	GSH
Control	5.38 ±0.13 b	302.28 ±16.86 B	**14.07** ±0.28 B	**64.96** ±2.39 B	**54.08** ±0.83 b
Diazinon	**3.29** ±0.24 a	**180.87** ±6.87 a,c	**12.96** ±0.21 a,c	**87.90** ±3.15 a,c	**59.84** ±1.31 a
Alpha-cypermethrin	**4.41** ±0.17 c	**175.69** ±10.01 a,c	**12.19** ±0.42 a,c	**82.63** ±4.17 a,c	**56.18** ±1.63 c

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)

3.2. Brain oxidative stress biomarkers

Brain superoxide dismutase (SOD) activity of DIZ and α-CYP exposed rats is shown in Table (2). A significant decline in brain SOD activity to 93.3% of control values in brain ($P < 0.05$) of α-CYP treated rats while, nonsignificant decrease DIZ exposed rats. Results in Table (2) indicated that CAT

activity in brain of DIZ supplemented rats exhibited a marked reduction reached to 85.6% compared with control group. However, CAT activity in rats supplemented with α-CYP exhibited a no significant decrease. As well as, brain GST activity was significantly increased in both DIZ and α-CYP administered rats to 122.8 % and 127.8% respectively. With respect to brain MDA levels comparing to the control group (Table 2) a significant increase was noticed with 163.2% and 151% in DIZ and α-CYP groups respectively. The results also revealed significant reduction in brain GSH content reached 90.1% and 91.1% in DIZ and α-CYP exposed rats comparing with control group. Finally, the present study showed a significant increase in malondialdehyde (MDA), a marked reduction in GSH levels As well as, enzymatic antioxidants (GST, CAT, SOD) in brain of male albino rats exposed to DIZ and α-CYP.

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	CAT	GST	MDA	GSH
Control	**5.42** ±0.08 b	**39.55** ±1.54 B	**1.40** ±0.09 b	**10.46** ±0.59 B	**61.21** ±0.56 b
Diazinon	**5.25** ±0.12 a,b	**33.86** ±0.30 a,c	**1.72** ±0.10 a,c	**17.07** ±1.073 a,c	**55.17** ±1.41 a,c
Alph-cypermethrin	**4.89** ±0.18 a,c	**36.75** ±1.03 a ,b,c	**1.79** ±0.06 a,c	**15.79** ±1.53 a,c	**55.76** ±0.85 a,c

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)

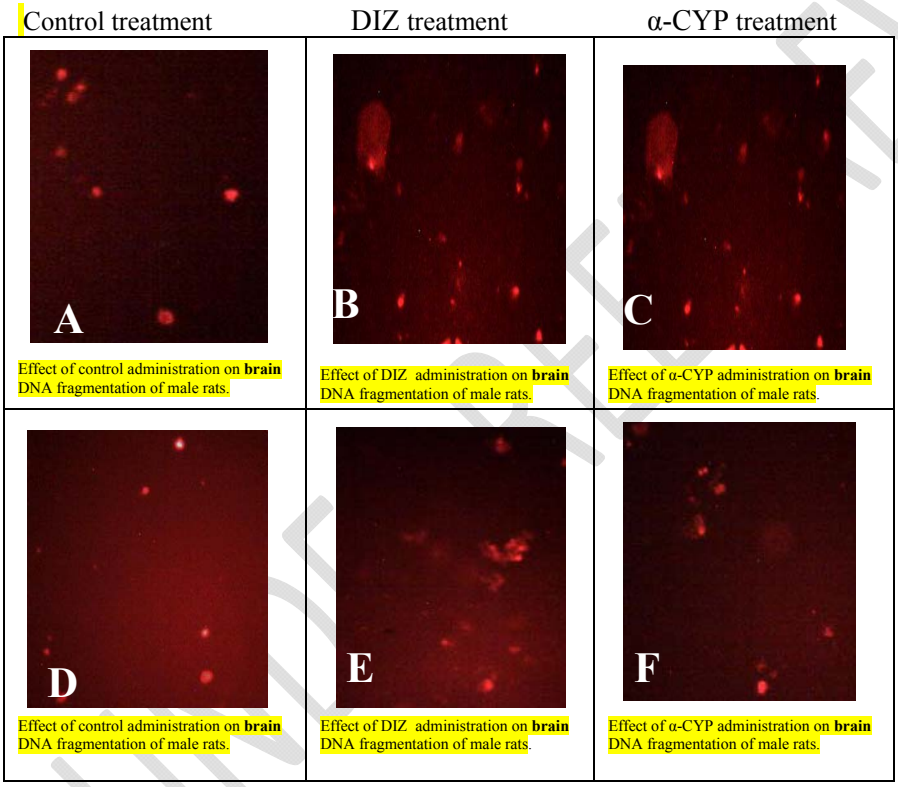
3.3. DNA damage

The DNA damages measured as percentage tail DNA in liver and brain tissues of DIZ and α-CYP- treated rats compared with control group are presented in Tables (3) and Fig. (1). The results showed that DNA damage was significantly increased in liver and brain tissues of exposed male rats to both insecticides as evidenced by an increase in both tail length and moment and increase in tail DNA%. With respect to control group. In coclusion The present study showed DNA damage indicating a genotoxicity in the liver and brain of male albino rats exposed to DIZ and α-CYP.

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		Tail DNA	
	liver	Brain	liver	Brain	liver	Brain
Control	1.60 ±0.04 b	0.98 ±0.05 B	5.07 ±0.19 b	4.23 ±0.07 b	29.16 ±0.92 b	20.13 ±0.47 b
Diazinon	6.24 ±0.39 a	2.94 ±0.24 A	11.87 ±0.34 a	8.7 ±0.86 a, c	46.88 ±0.94 a	37.58 ±1.59 a
Alpha-cypermethrin	4.57 ±0.30 c	5.36 ±0.47 C	9.87 ±0.32 c	10.23 ±0.73 a, c	40.17 ±1.37 c	44.79 ±1.63 c

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)



Effect of control administration on **brain** DNA fragmentation of male rats.

Effect of DIZ administration on **brain** DNA fragmentation of male rats.

Effect of α-CYP administration on **brain** DNA fragmentation of male rats.

Effect of control administration on **brain** DNA fragmentation of male rats.

Effect of DIZ administration on **brain** DNA fragmentation of male rats.

Effect of α-CYP administration on **brain** DNA fragmentation of male rats.

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.

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. 2-5) control group showed the normal histological structure of hepatic lobules. (Figs. 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).

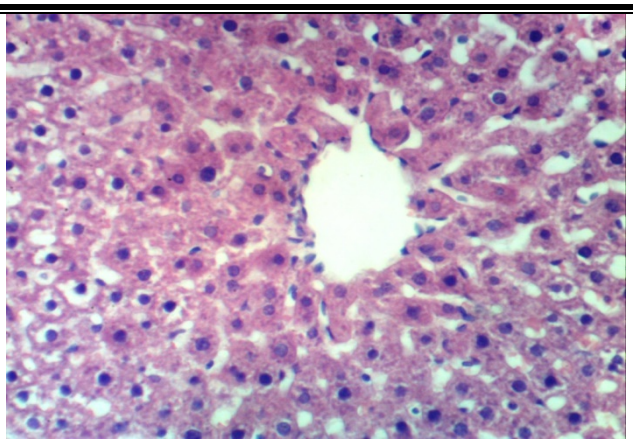


Fig. 2: Microscopical examination liver of rat from control group showed the normal histological structure of hepatic lobules.

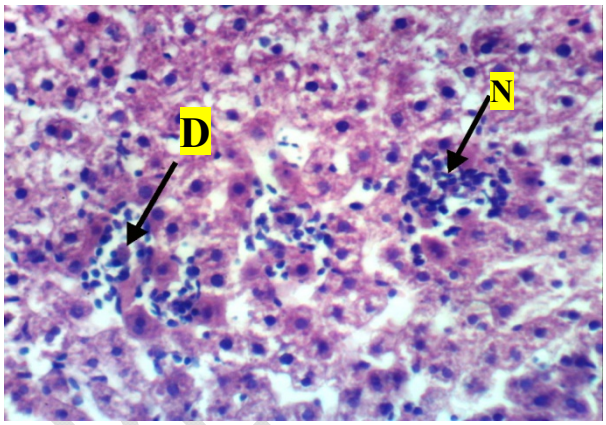


Fig 3: Microscopical examination of liver of rat from DIZ group revealed D=hydropic degeneration of hepatocytes and multiple focal N=hepatic necrosis associated with inflammatory cells infiltration.

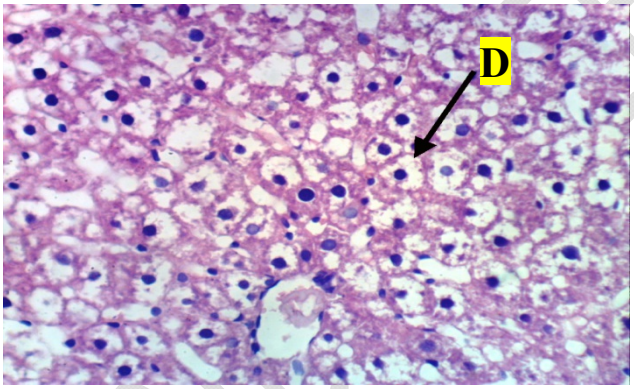


Fig.4 : Microscopical examination in examined liver of rats from α-CYP group revealed D=hydropic degeneration of hepatocytes.

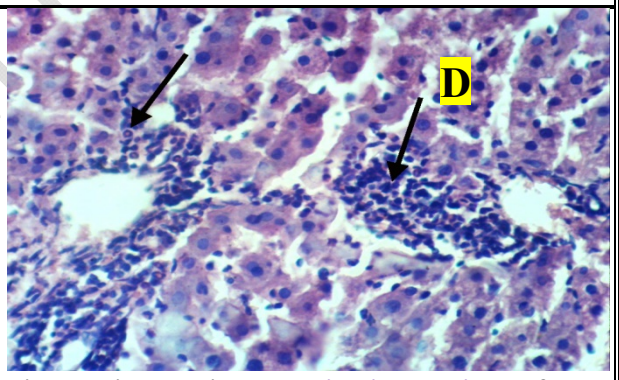


Fig.5: Microscopical examination in liver of rats from α-CYP group revealed D=hydropic degeneration of hepatocytes.

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 control group revealed no histopathological changes. (Fig. 6), necrosis of neurons, neuronophagia (Fig. 7), focal gliosis (Fig. 8) and cellular oedema (Fig. 9). While, brain tissues in α-CYP-treated group revealed congestion of cerebral blood vessel (Fig. 10), cellular oedema (Figs. 11) and focal gliosis (Fig. 12).

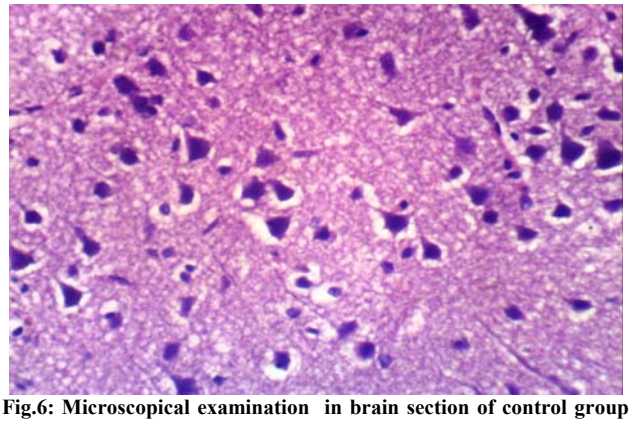


Fig.6: Microscopical examination in brain section of control group revealed no histopathological changes.

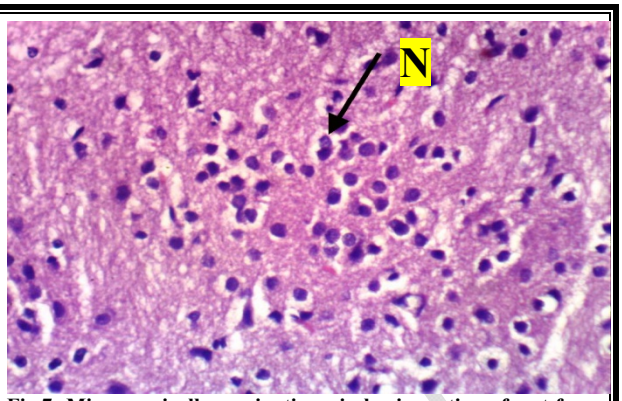


Fig.7: Microscopicall examination in brain section of rat from group DIZ has a N=necrosis of neurons and neuronophagia

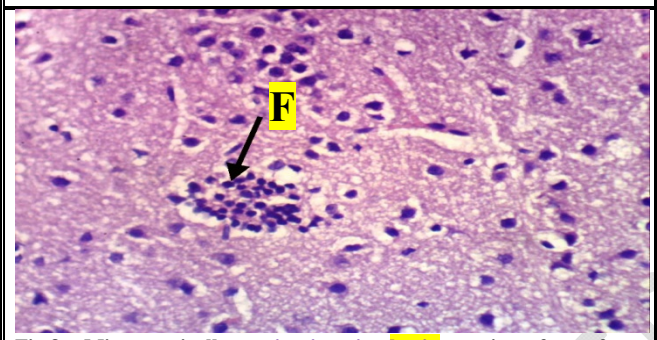


Fig.8: Microscopicall examination in brain section of rat from group DIZ has focal gliosis

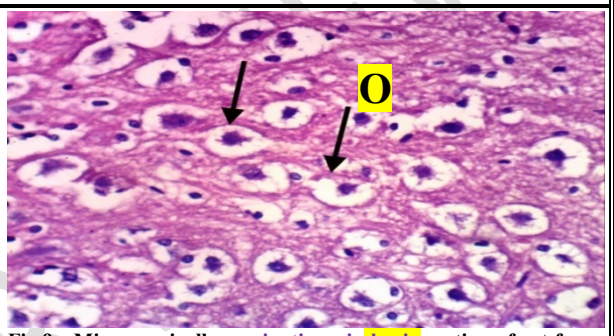


Fig.9: Microscopicall examination in brain section of rat from group DIZ has cellular oedema.

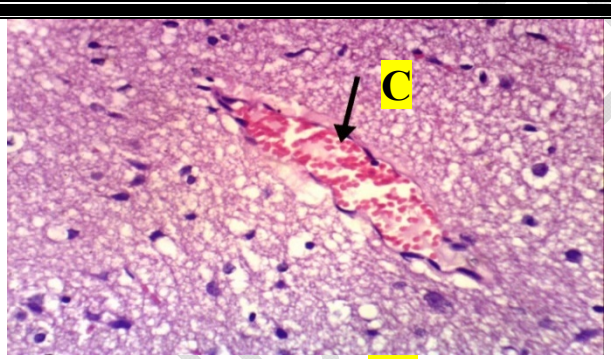


Fig.10: Microscopicall examination in brain section of rat from group α-CYP revealed C=congestion of cerebral blood vessel.

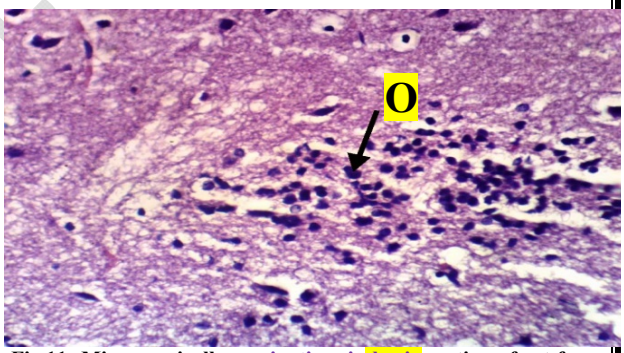


Fig.11: Microscopicall examination in brain section of rat from group α-CYP O =cellular oedema.

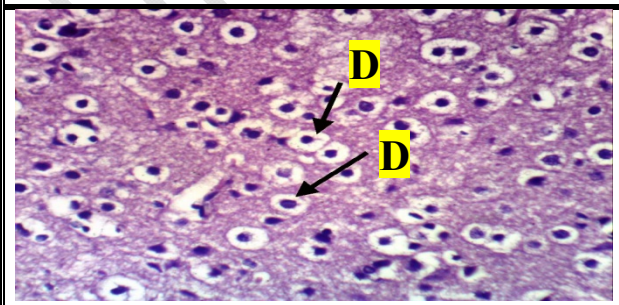


Fig.12: Microscopicall examination in brain section of rat from α-CYP showed focal gliosi.

Discussion:

Since, oxidative stress is involved in diseases processes and inflammatory response as a promoter of cellular pathways; it has been in the first mechanistic studies of pesticides exposure (Singh *et al.,* 2007). Metabolism and bioactivation of xenobiotics are a major source of increased free radical formation that, implicated in cellular injury or physiological dysfunction, Pesticides exposure are one of the causes of increased oxidative stress level, and it may result in altered disease susceptibility(Rehman *et. al.,* 2014).

Lipid peroxidation (LPO) is considered a potential hazard of pesticides toxicity as many environmental chemicals are linked with over production of reactive oxygen species (ROS) that attack polyunsaturated fatty acid in cell membrane leading to enhancement of LPO (Halliwell, 2007). MDA is an important biomarker of lipid peroxidation occurred in polyunsaturated fatty acids (Demir *et al*., 2011). The mechanisms of the cytotoxic effects of some pesticides are linked with Lipid peroxidation of polyunsaturated fatty acid residues that occurs in phospholipids-basic components of cell membranes (Marnett, 2002). The disruption in phospholipids components of cell causes a cytoplasmic and mitochondrial membrane damage that led to increases in the production of free oxygen radicals within the cells (Kang and Hamasaki, 2003)

GSH plays a normal balance between oxidation and anti-oxidation and regulates the vital functions of cells such as the synthesis and repair of DNA, the synthesis of proteins and the activation, maintaining the essential thiol status of protein (Hogg, 2002). GSH and GSH-related enzymatic systems in cells drive benefit roles in detoxification and have the most physiological role. GSH is a nucleophile that can react with electrophilic species rendering the electrophilic molecules more solubility and unable to interact with cellular constituents

(Aquilano *et al.,* 2014*).* Reduction in GSH levels may red uce the cellular power for destroying free radicals and reactive oxygen species. Depletion of glutathione may be linked with induction of GST activity where, GST-mediated conjunction considered an important mechanism for detoxifying lipid peroxidation products (Leaver and George, 1998).

Organophosphates caused a significant increase in MDA (lipid peroxidation end product) level and a decrease in antioxidants (GSH) content (Amal *et al*., 2005). Lipid peroxidation and decreased total antioxidant capacity in rat liver of diazinon exposed rats were noticed in previous study of (Amirkabirian *et al.,* 2007) & (Abdou and El-Mazoudy, 2010). Which have a number of adverse biological effects by interfering with normal cell homeostasis in several ways including blocking protein metabolism (Grune *et al*., 2004), and interference in GSH pathways (Lu, 1999). 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 level (Elia *et al.,* 2008).

SOD destroy the free radical superoxide anion into molecular oxygen and hydrogen peroxide and prevents formation of hydroxyl radicals and plays an important role in the cellular antioxidant mechanism (sharma *et al.,* 2007).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 (Santiard *et al*., 1995). Also, SOD activity reduction reflects oxidative stress (Jenkins and Goldfarb, 1993). Superoxide dismutase activity is modulated by both tissue oxygenation and generation of ROS (Pigeolet *et al*., 1990). CAT is involved in a variety of biochemical functions; breakdown of high levels of H_2O_2 and its removal (Schneider, and de Oliveira, 2004). The reduction in CAT activity may be attached to the tested pesticides 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 have been shown to inhibit CAT activity (Kono and Fridovich, 1982) as, O_2 oxidize the ferrous state of CAT and led to CAT inactivation (Freeman and Crapo, 1982). The decrease of cellular antioxidant defense mechanisms reflected the inability of tissue to scavenge excess ROS, which could be served as one of the reasons of ROS increase **(**Sun *et al*., 2009**).**

Any DNA damage such as strand breaks may be linked with cell integrity disruption consequently cell toxicity or cell death at the end (Bedir *et al*., 2008). DNA lesions with physiological relevance to neural cells are single strand DNA breaks, which arise from the disintegration of the sugar phosphate backbone of DNA following oxidative attack by ROS (Brochier and Langley, 2013). Strand breaks are expressed in DNA during sugar fragmentation events and when excision repair enzymes remove damaged bases (Imlay and Linn, 1988). Furthermore, ROS may indirectly damage DNA via the production of oxidized lipid and protein byproducts that form adducts (Moller and Wallin, 1998).

Synthetic pyrethroids are lipophilic insecticides that affect lipid constituents in lipid packing in cell membrane and lead to disturbance in cell membrane **(**Rehman *et al*., 2014**).** Cypermethrin hydrolysed via hydrolytic ester cleavage and metabolized by the CYP-450 enzymes leading to ROS (Klimek, 1990). This over production in ROS mediated lipid oxidation and Ca^{++} release from storage in sarcoendoplasmic reticulum and consequently cytotoxicity and genotoxicity in vertebrates during exposure (Kale *et al*., 1999). Lipid peroxidation may be enhanced by free radicals generated by pyrethroids and this may be the mechanism of pesticides toxicity (Valavanidis *et al*., 2006).

DNA fragmentation observed in the present study is the normal consequence of oxidative stress that was demonstrated through elevation in LPO, reduction in antioxidant enzymes such as and glutathione content in rat liver. This is also consistent with previous studies where DNA fragmentation was induced by lambda cyhalothrine in rat lymphocytes (Sharma *et al*., 2010) and by cypermethrin in rat brain (Hussien *et al*., 2011).

Pathological examination of liver in diazinon and alpha cypermethrin exposed rats revealed hydropic degeneration of hepatocytes and multiple focal hepatic necroses associated with inflammatory cells and portal infiltration. Inflammatory cells act as a defense mechanism to

toxic materials. Similar results were observed by (El-Shenawy, 2009) who found that mice intoxicated with diazinon resulted in hydropic degeneration and necrosis 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, congestion of cerebral blood vessel. Microglial cells have receptors that enable them to sense damaged tissue and to recognize viruses , environmental and endogenous toxins and other pathogens. Such recognition leads to upregulate (activate) of microglial cells. (Activated microglial encircle degenerating neurons (neuronophagia)). Gliosis is a nonspecific reactive change of glial cells in response to damage the central nervous system (CNS). In most cases, gliosis involves the proliferation or hypertrophy of several different types of glial cells, including astrocytes, microglia, and oligodendrocytes.

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 the various membrane components of the cell (Stal and Olson, 2000), or may be attributed to liver damage resulting in a decrease in antioxidant defenses in the liver (Abdollahi, *et al.,* 2004).

Conclusion and Recommendations:-

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

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