Oral and dermal exposure of chlorpyrifos and cypermethrin mixture induced cytogenetic, histopathological damage and oxidative stress in rats

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Abstract: Present study is aimed to estimate the risk and the hazard of oral and dermal exposures of chlorpyrifos and cypermithrin mixture which are commonly used in Egypt. And to evaluate which type of exposure is more toxic or dangerous than other. Subacute toxicity of repeated 28 days in male white rats was assessed. The study was included some toxicological parameters ranged from cytogenetic, histopathological and oxidative stress effect. Eighty Wistar strain rats (weighing 140-160g) were randomly divided into four main groups (a, b, c and d) which were further subdivided into subgroups. Each of group (a) and (b) consists of 5 subgroups and were used to determine the oral and dermal LD_{50}. Each of group (c) and (d) consists of 3 subgroups and were used in oral and dermal treatments respectively. The first subgroups of (c) and (d) were kept as control. Rats of two subgroups (c) treated orally by gavage 1/20 and 1/30 of LD_{50}. Rats of two subgroups (d) treated dermally by 1/30 and 1/50 of LD_{50}. The results revealed that, the pesticides mixture induced neurotoxicity, genotoxicity, severe histological changes. Also resulted in lipid peroxidation, inhibition in the activities of antioxidant enzymes (Cat) and reduced the glutathione contents. In addition the results showed the dermal treatments were more toxic and hazard than oral treatments. So, these mixtures of pesticides when present together may induce bio-activation sites resulting in the increase of the observed toxicity of these pesticides mixture compared with the toxicity of the individual alone. Finally, the results strongly impose the need to more detailed testing of the toxicity of mixture exposure than to one individual.
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Key words: chlorpyrifos, cypermethrin, mixture, Rats, chromosomal aberrations, Histopathology, oxidative stress.

1. Introduction

The widespread use of pesticides in public health protection and agricultural pest control has caused severe environmental pollution and health hazards, especially in developing countries, including cases of severe acute and chronic human poisoning as well as damage to other non-targeted organisms. Every year there are 3 million cases of severe poisoning and 220,000 deaths from pesticides; the majority of these poisonings approximately 99% of death cases occur in the third world **(Tinoco *et al.*, 1998)**. In addition most of the pesticide sprayers in Egypt are not protected by safety measures when using the pesticides in the fields; therefore they are directly exposed to these compounds, whose toxicity is ranged from moderate to hazardous **(Mansour, 2004).**

Many pesticides have been tested for toxicological studies. A limited number of these studies have been done to evaluate the risk of mixture of more than one active ingredient. Now the large scale of using these mixtures of pesticides has been increased in developing countries **(Committee on Toxicity of Chemicals, 2002)**. The introduction of these new mixtures into the environment has necessitated accurate identification of their potential hazards to human and animal health **(Bolognesi, 2003)**. In addition the local manufacturers formulate a mixture of pesticides ready to use. These mixtures may be more hazards than using each of their alone. On the other hand the different routes of exposure give different degree of risk. The oral and dermal exposure may be the major routes of entry of pesticides that can happen in Egypt because the Egyptian spray workers do not care to use the safety measures of pesticides.

This study was conducted to estimate a new pesticide formulation (Chlorosan 29% EC) which is local made and sale as pesticide ready to use in agricultural pest control in Egypt. This mixture may be included some second products or impurities which are unknown. So study the toxicity of this new mixture is very essential and necessary for risk assessment. The pesticide formulation consists of two traditional pesticides chlorpyrifos and cypermethrin. Cypermethrin is widely used as insecticide in developing countries to control a wide range of insects, especially Lepidoptera. It is recently back to use in agriculture after it banned for several years in Egypt. Human exposure to cypermethrin is reported

to occur mainly occupationally during application or through pyrethroids residues such as those detected in cow's milk, bread, fruits and vegetables (**Sankar *et al.*, 2010**). In an epidemiological study, population exposed to cypermethrin in cotton fields showed ill health effects such as severe giddiness, nervous, skin and eye disorders, neonatal deaths and congenital defects. Cypermethrin can also elicit a range of neurotoxic, immunotoxic and genotoxic effects and reproductive toxicity in various experimental systems **(Yousef *et al.*, 2003)**. Cypermethrin caused an increase in the number of cells with abnormal chromosomes in both bone marrow and spleen **(Institoris *et al.*, 1999)**. All of the above mentioned make cypermethrin is one of the most common contaminants in the ecosystem. On the other hand Chlorpyrifos is one of organophosphate (OP) insecticides which are still widely used for agriculture, pest control and domestic purposes. Thus the uncontrolled application of these insecticides in agriculture and public health operations increased the scope of ecological imbalance and thus many non-target organisms have become victim **(Ojha *et al.*, 2011)**. Organophosphate (OP) compounds including Chlorpyrifos are known to inhibit acetyl-cholinesterase and pseudocholinesterase in target tissues resulting in accumulation of acetylcholine in synaptic junctions **(Shenouda *et al.*, 2009)**. This excessive accumulation of acetylcholine in synapses leads to activation of cholinergic, muscarinic and nicotinic receptors and hyperactivity in the cholinergic pathways. However, AChE inhibition does not explain all the symptoms of OP intoxication. Other systems that may be affected by OP exposure are the immune system **(Galloway and Handy, 2003)**. Hematological system **(Jintana *et al.*, 2009)**, and reproductive system **(Farag *et al.*, 2000** and **Uzun *et al.*, 2009)**. OP pesticides have demonstrated genotoxic, alkylating and clastogenic properties; thus they are potentially mutagenic and clastogenic **(Mehta *et al.*, 2008)**. More recently, it has been postulated that OP pesticides produce oxidative stress in different tissues through the formation of reactive oxygen species (ROS) **(Akhgari *et al.*, 2003**; **Abdollahi *et al.*, 2004**; and **Mehta *et al.*, 2009)**.

So, the aim of this work was to verify the side effects of this mixture of two pesticides and evaluate which routes oral or dermal is more hazards than other. This study was included some parameters that covered some toxicological points ranged from cytogenetic, histopathological and oxidative stress effects.

2. Materials and Methods

2.1 The pesticide tested

The pesticide tested used in this study was a mixture of two active ingredients consisted of cypermethrin 5%, the IUPAC name: (*RS*) - α-cyano-3-phenoxybenzyl (1*RS,* 3RS; 1RS, 3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and chlorpyrifos 24%, the IUPAC name: (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) The formulation was supplied as an emulsifiable concentrate (EC) as well by the Department of Mammalian and Aquatic Organisms Toxicity Research, Central Agricultural Pesticides Lab, Dokki, Egypt. A stock solution (10%) of the formulation was prepared in corn oil and was used throughout the oral treatments but the dermal treatments were used as it is without diluted.

2.2 Animals

A total of 80 apparently healthy male white rats (*Rattus rattus*) of Wistar strain weighing 120g+10% were used throughout the whole work. The animals were obtained from the laboratory animal house of the Modern Veterinary Office, Giza, Egypt. Animals were kept under full hygienic conditions, had free access to fresh water and fresh well-balanced food, and remained under supervision for two weeks before commencing the experimental work. The animals were housed in all groups of five rats per cage.

2.3 Determination of the median lethal dose, LD_{50}

The median lethal dose (or LD_{50}) is defined as the dose of a test substance that is lethal for 50% of the animals in a dose group. This test should perform to provide preliminary information on the toxic nature of a pesticide tested for which no other toxicology information is available for this new mixture. The oral and dermal LD_{50} were determined by using classical method according to **Weil (1952).**

2.4 experimental design

Rats were randomly divided into four main groups (a, b, c and d) which were further subdivided into subgroups. Each of group (a) and (b) consists of 5 subgroups and were used to determine the oral and dermal LD_{50}. Each of group (c) and (d) consists of 3 subgroups and were used in oral and dermal treatments respectively. The first subgroups of (c) and (d) were kept as control. Rats of two subgroups (c) treated orally by gavage 1/20 and 1/30 of LD_{50}. Rats of two subgroups (d) treated dermally by 1/30 and 1/50 of LD_{50}. Rats were treated for 28 consecutive days.

2.5 Blood collection and tissue preparation

Rats were sacrificed through cutting of their neck veins at the end of treatments after they were anaesthetized by ether. Blood samples were collected from the sacrificed animals and placed immediately

on ice. Heparin was used as an anticoagulant and plasma samples were obtained by centrifugation at 1,100 rpm for 20 min and plasma samples were used immediately for analyzing the oxidative stress parameters. Liver was immediately removed and washed using chilled saline solution. Half of liver tissues were preserved in formalin (10%, w/v) for histological parameter and another part served to the biochemical parameter. Both femurs were dissected out and prepared to Chromosomal aberrations assay.

2.6 Biochemical parameters

2.6.1. Glutathione Reduced (GSH)

The method based on the reduction of 5,5'-dithio-*bis*-2-nitrobenzoic acid (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm. Briefly fresh heparinized blood sample was used and the erythrocytes (red blood cells) were lysed by add 4 fold volume cold distilled water then centrifuged at 4,000 rpm for 15 min. the supernatant was collected and added to the reagent contain (DTNB) then mixed well and measured the absorbance after 5-10 min. the method according to **(Beutler and Kelly, 1963)**.

2.6.2. Catalase (CAT)

Catalase (CAT) activity was estimated by the method of **Aebi *et al.* (1984)**. The method based on the Catalase reacts with a known quantity of H_2O_2 and the reaction is stopped after exactly one minute with catalase inhibitor. Briefly the plasma were collected as above mentioned and added to the reaction mixture containing 0.8 ml phosphate buffer (K_2HPO_4/NaH_2PO_4, 50 mM, pH 7.0), and 0.1 ml triton X-100 (0.02%) then incubated at room temperature for 10min. Reaction was initiated by addition of 2.0 ml H_2O_2 (0.03 M prepared in potassium phosphate buffer, pH 7.0) and absorbance change per min was recorded for 5min at 240nm. Specific activity is expressed as 1 mole H_2O_2 decomposed min^{-1} mg^{-1} protein.

2.6.3. Lipid peroxidation

The end-products of lipid peroxidation, is malondialdehyde (MDA) which reacts with thiobarbituric acid reactive substances (TBARS) to yield a fluorescent product so the most commonly used test is called (TBARS) assay **(Okhawa *et al.*, 1979)**. Thiobarbituric acid (TBA) reacts with (MDA) in acidic medium at temperature of 95°C for 30min to form thiobarbituric acid reactive product the pink product can be measured at 534 nm. A 10% (w/v) of liver homogenate prepared in 1.15% KCl is a tissue sample used in this assay. The levels of MDA are expressed as nmol g^{-1} tissue.

2.6.4. Plasma cholinesterase

Plasma cholinesterase or butyrylcholinesterase (BuChE) activity was estimated by the method of **(Ellman *et al.*, 1961)**. The reaction mixture containing 2.6 ml sodium phosphate buffer (0.1 M, pH 7.5), 0.15 ml of DTNB (dithio-bis-2-nitrobenzoic acid, 10 mM, pH 7.0; containing 3 mg $NaHCO_3$ per 8 mg DTNB) and 0.1 ml plasma was incubated at room temperature for 10 min. Reaction was initiated by addition of 0.15 ml acetylthiocholine iodide (12.5 mM) and absorbance change per min was recorded at 412nm for 4min. Specific activity is expressed as nmoles product formed min^{-1} mg^{-1} protein using molar extinction coefficient of the adduct formed between thiocholine and DTNB as 13600 M^{-1} cm^{-1}.

2.7. Histological section preparation

Liver specimens were obtained from rats, and immediately fixed in 10% formalin for 24 hr and decalcification was occurred on formic acid then washed in tap water. Serial dilutions of alcohol (absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 hrs. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by Hematoxylin & Eosin stain then examination was done through the light microscope **(Banchroft *et al.*, 1996).**

2.8 Cytogenetic investigations

Cytogenetic analysis of rat chromosome aberrations (CA) in bone-marrow metaphase cells was performed according to the technique described earlier by **(Adler, 1984)** with some modifications as recommended by authors. Animals were intra-peritoneal injected with colchicines (4mg/kg bw) 2 hrs prior to the scheduled time of sacrifice, in order to accumulate metaphase cells and provide more readily analyzable chromosomes. The rats were sacrificed 24hrs after the last dose treatment. Both femurs were dissected out and cleaned of any adhering muscle, the bone-marrow cells were collected from both femurs by flushing with Hank's balanced salt solution (HBSS) then centrifuged at101×g for 10min and the was re-suspended very well with a potassium chloride hypotonic solution (0.075 M KCl) and after that the tubes were kept in the refrigerator at 4°C for 45-min. This step is very important (modified and recommended by the authors).The cell suspension was centrifuged at 101×g for 10min, fixed in methanol: glacial acetic acid, 3:1, v/v).

Centrifugation and fixation (in the cold) were repeated three times at 20min intervals. The material was re-suspended in a small volume of fixative, dropped on to chilled slides, flame-dried, and stained in the next day with 20% Giemsa. One hundred good-quality metaphases containing 42 chromosomes were examined per animal to score different aberrations.

2.9 Statistical analysis

The data obtained in this study were calculated and statistically analyzed, according to Studen's t-test **(Venables and Ripley, 2002)**, using statistical software.

3. Results

3.1. Results of median lethal dose

The results of oral and dermal LD50 are illustrated in table (1) and the doses used in the study were determined according to the values of LD_{50} of both oral and dermal treatments.

Table(1): median lethal dose (LD_{50}) estimated on male rats

Treatments	LD_{50} (mg/kg)	Doses used in the study (mg/kg) Low dose	High dose
Oral	85.28	2.84 (1/30 LD_{50})	4.26 (1/20 LD_{50})
Dermal	2125.64	42.51 (1/50 LD_{50})	70.85 (1/30 LD_{50})

3.2. Effects on the cytogenetic parameters

The frequency of chromosomal aberrations in rats bone marrow induced by each type of treatments is shown in Table (2) and images of some types of aberrations obtained from our results are illustrated in figure (1). The obtained data of the oral treatments showed significant increase (p<0.01) in the chromosomal aberrations (CAs) only at high dose (1/20 LD_{50}). But low dose (1/30 LD_{50}) did not induce significant induction in (CAs). In contrast to the oral treatments, the dermal treatments induced highly significant increase at high and low doses (p<0.001). So it is prominent that the dermal treatments were more genotoxic than oral treatments. On the other hand the most frequent aberrations were the centromeric attenuation (C-A) followed by chromatid gap (tg) and end to end association (E-E), while the chromtide break (tb) was the lowest aberration scored. Numerical aberrations which showed as a polyploidy (4n) were recorded in the negative control groups but the treatment groups increased the percentage of polyploidy percentage in all doses.

Table(2): Chromosomal aberrations induced in rats bone marrow after different treatments of pesticide tested

	Treatments	Types of structural aberrations tg	tb	E-E	C-A	Numerical aberration (polyploidy)	Total aberrant cells/ 250 scored metaphases	Mean ± S.E.
	[a] Control group	3	1	0	2	5	11	2.2 ± 0.663
Oral treatments	Low dose (1/30 of LD_{50})	4	0	3	5	8	20	4.0 ± 0.775
Oral treatments	High dose (1/20 of LD_{50})	7	1	5	9	9	31	6.2 ± 0.860 **
	[a] Control group	3	2	2	1	11	19	3.8 ± 0.374
Dermal treatments	Low dose (1/50 of LD_{50})	9	6	9	9	20	53	10.6 ± 0.510 ***
Dermal treatments	High dose (1/30 of LD_{50})	7	6	10	12	24	59	11.8 ± 0.735 ***

Values are from five replicates in each treatment and the last column represent mean ± S.E. of aberrant cells per 250 spread metaphases/treatment, *** Significant at $p < 0.001$; ** Significant at $p < 0.01$

[a] Control group, the rats were treated with corn oil

Abbreviations: **tg**, chromatid gap; **tb**,chromatid break; **E-E**, end to end association; **C-A**, centromeric attenuation.

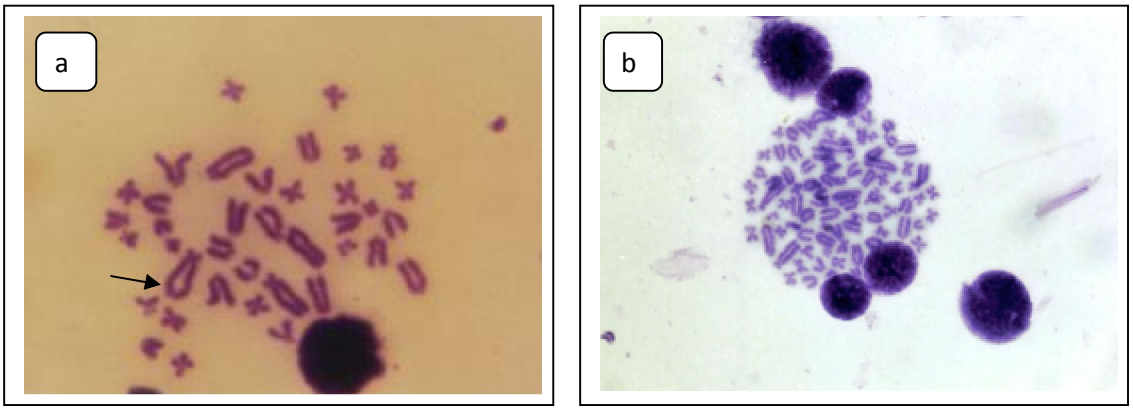

Fig (1) Rats bone marrow metaphases, showing (a) end to end association; and (b) polyploidy (4n)

3.3. Effects on antioxidant parameters

The results summarized in Table (3) indicated the treatment with the pesticide tested increased significantly the level of TBARS ($p < 0.001$) in both dermal and oral treatments as indication of increased the lipid peroxidation. But the GSH content and the activities of CAT were significantly decreased at high dose in dermal treatment only ($p < 0.001$). And oral treatments did not show significant different. On the other hand, the pesticide tested decreased with highly significant level of plasma cholinesterase BuChE ($p < 0.001$) at all treated doses.

Table (3): The activities of CAT, BuChE, and the levels of GSH in blood and TBARS in liver of rats treated with pesticide tested

Parameters	Experimental groups				
		Oral treatments		Dermal treatments	
	Control group	low dose (1/30 of LD_{50})	high dose (1/20 of LD_{50})	low dose (1/50 of LD_{50})	high dose (1/30 of LD_{50})
GSH(mg/dL)	19.63±0.46	19.60 ± 0.58	17.09 ± 0.73	20.33 ± 0.51	13.08 ± 0.60***
CAT (U/L)	351.54 ± 18.59	429.79 ± 40.75	291.05 ± 24.81	173.58 ± 11.60***	202.3 ± 17.05***
TBARS (mg/g.tissue)	6.57±0.64	10.55 ± 0.74***	11.77 ± 0.43***	18.39 ± 0.46***	12.19 ± 1.54***
BuChE (U/L)	398.58 ± 9.7	174.30±8.37***	203.56 ± 3.87***	140.62±4.13***	159.18±6.57***

*** Significant at $p < 0.001$

3.5. Histological examination of the liver

Histopathological examination of specimens taken from both control groups of oral and dermal treatments are showed in Fig.2 (A-1 & B-1) respectively. And the oral-treated groups showed in the central veins and sinusoids much congestion associated with apoptosis in some of the surrounding hepatocytes (A-2 & A-3). Inflammatory cells infiltration was detected in few manners in between the hepatocytes (A-4). But the dermal-treated groups more congestion and Sever dilatation were detected in the central and portal veins as well as the hepatic sinusoids (B-2). Also Fatty change was detected in the hepatocytes associated with inflammatory cells infiltration and pigmented material in between (B-3 & B-4).

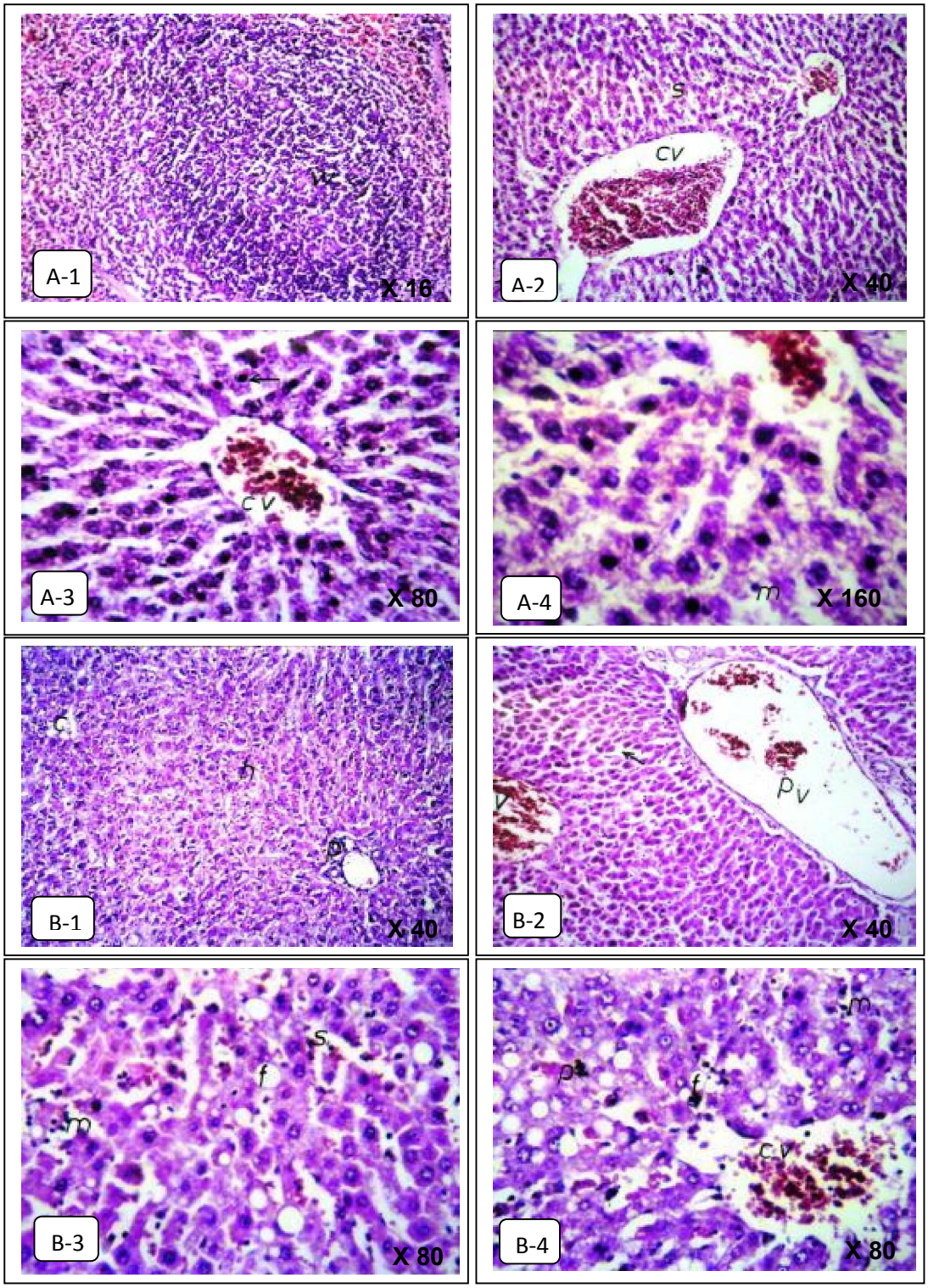

Fig (2). Light micrographs of the liver tissue of rats treated as follow: groups (A) of oral treatments (A-1, normal tissue), (A-2, showing congestion in central vein), (A-3, showing apoptosis ←), (A-4, showing few Inflammatory cells infiltration), and groups (B) of dermal treatments (B-1, normal tissue), (B-2, showing Sever dilatation and congestion in central and portal veins), (B-3, showing fatty change in the hepatocytes associated with inflammatory cells infiltration), (B-4, showing pigmented material in between the hepatocytes). Tissue sections were stained with H&E.

4. Discussion

4.1. Effects on the acute toxicity

The acute toxicity of the mixture of pesticides (chlorpyrifos and cypermethrin) increased the toxicity of each pesticide as individual because the oral LD_{50} of chlorpyrifos and cypermethrin for rats are 135-163 and 250-4150 mg/kg respectively **(Pesticides manual, 2004).** But the estimated acute oral LD_{50} of formulation tested which consists of both these active ingredients is 85.28 mg/kg. Also dermal LD_{50} of chlorpyrifos or cypermethrin for rats is more than 5000 mg/kg. But the estimated dermal LD_{50} is 2125.64 mg/kg. So it is obvious that the mixture of pesticides may made synergistic effect of acute toxicity. These results are in agreement with **(Cathy *et al.,* 2009)**.

4.2. Effects on the cytogenetic parameter

Genotoxic effects are considered among the most serious of the possible side effects of agricultural chemicals. The prolonged exposure to such chemicals may lead to effects including heritable genetic diseases, carcinogenesis and birth defects **(Patel *et al.,* 2007)**. In spite of many previous findings reported that there is no indication of genotoxic activity for chlorpyrifos in many genotoxicity assays in both mice and rats **(Gollapudi *et al.,* 1995)** our data showed significant increased in (CAs) at high oral dose and both dermal doses, this may be due to that one of the components of the pesticide tested has genotoxic effect than other. The finding that support this investigation the other component of the pesticide mixture, cypermethrin which has hydrophobic nature and small molecular size can passes through the cell membrane and reaches the nucleus. It is suggested that within the nucleus it binds to DNA through the reactive groups of its acid moiety, leading to destabilization as well as unwinding of the DNA, which could be a possible mechanism for its genotoxicity **(Saxena *et al.,* 2005)**. Moreover, cypermethrin has both a vinyl and a dimethylcyclopropane group. Dimethylcyclopropane may be oxidized into methyl butenol via the rearrangement of radical and the formation of carbocation. If the vinyl group and/or the methyl butenol group undergo epoxidation, the resultant active metabolites may cause DNA damage **(Sankar *et al.,* 2010)**. Cypermethrin has also been shown to induce oxidative stress and generation of reactive oxygen species (ROS) in experimental systems. It has been demonstrated that ROS may cause DNA damage, which could lead to single-strand breaks and mutation **(Woo *et al.,* 2009)**. In addition the difference in potential mutagenicity of cypermethrin *in vitro* and *in vivo* studies could be due to the fact that under *in vivo* system metabolites generated could be more reactive to DNA leading to increased genotoxicity **(Patel *et al.,* 2007)**. Also **Gabbianelli *et al.,* (2004)** suggested that superoxide anion and hydrogen peroxide are the main source of cypermethrin-induced free radical production, and this is coincided with the present results. This provides another probabiltiy that DNA damage observed after cypermethrin exposure could be a consequence of free radical attack. Thus, the increase in lipid peroxidation and depletion of antioxidant enzymes play a major role in cypermethrin-induced genotoxicity.

4.3. Effects on the Oxidative stress parameters

Oxidative stress is unbalance between the production of free radicals and antioxidant defenses in the body; as it can results in oxidative damage to lipids (lipid peroxidation), proteins, carbohydrates, and nucleic acids. In most cases, the abnormal generation of reactive oxygen species (ROS), which results in significant damage to cell structure, is considered an important signal of oxidative damage **(Barzilai and Yamamoto, 2004)**. Several xenobiotics and environmental pollutants are known to cause this imbalance between formation and removal of ROS. Obtained results showed that the combination of two active ingredients (chlor & Cyp) in formulation tested generated oxidative stress in rat tissues as evidenced by the elevation in TBARS, the reduction in the antioxidant enzymes (CAT) and glutathione (GSH) content in rat tissue. This is might be attributed to the metabolic activation of cypermethrin, which is considered a major mechanism of its toxicity. Cypermethrin caused significant oxidative stress in liver tissue of rats as was evident by the elevation of the level of TBARS. Reduced level of total glutathione also indicated the occurrence of an oxidative insult **(Giray *et al.,* 2001)**. Cypermethrin can be expected to have two modes of action: it may induce oxidative stress by generate (ROS) or, as a hydrophobic compound it may accumulate in cell membrane and disturb membrane structure.

On the other hand, OPs are known to produce oxidative stress by increased generation of ROS and decreased levels of cellular antioxidants **(Saxena, 2010)**. The ROS may be produced as a result of the metabolism of Ops by cyt P450. The cyt P450s are monooxygenases and catalyze oxidation by addition of one atom of molecular oxygen into the OP compounds at electron transport pathway. Also OP pesticides induced peroxidative damage of membranes and accumulation of lipid peroxidation products in cells, tissues and serum of rats, these have been reported by **(Institoris *et al.,* 1999** and.**Sankar *et al.,* 2010)**

More addition, Antioxidant enzymes namely catalase (CAT), and other enzymes are the first line of defense against oxidative stress. It catalyzes the decomposition of hydrogen peroxide to water and oxygen **(Chelikani *et al.*, 2004)**. It is a very important enzyme in reproductive reactions. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second **(Goodsell, 2010)**. Decrease in the activity of this enzyme change the following induction of oxidative stress **(Choi *et al.*, 2009)**. Obtained data of the present study showed decrease in the activities of CAT in plasma of treated rats in only dermal treatments but oral treatments did not show this effect. So CAT seems to be inhibited by chlorpyrifos & cypermethrin at high dose levels and it could be attributed in part to the damaging effect of these treatments on liver cells as confirmed by the damaging observed in histological examination in the liver tissue. These investigations are agreement with previous findings; histo-pathological changes ranged from vacuolization and necrosis were found in rat liver tissue after chronic administration by cypermethrin **(Joshi *et al.*, 2007).** Also histological scoring of rats liver damage after were given a single daily oral doses of chlorpyrifos 1/20 LD50 for 28 consecutive days **(Heikal. et al., 2012).** Furthermore, in healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress. The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity. So glutathione protects cells from the free radicals produced through oxidation **(Pastore *et al.*, 2003)**. Our results showed highly significant decrease in (GSH) contents only at high dose of dermal treatments that is agreed with Singh ***et al.*, (2006)** reported reduced Glutathione (GSH) content of RBCs was decreased after treatment with the pesticides, and increased the activities of glutathione-s-transferase (GST) and glutathione reductase (GR) were due to induction of natural defense mechanism of elythrocytes against the toxicity of the pesticides. Altered activities of these enzymes along with decreased GSH content indicate increased oxidative stress in elythrocytes after treatment with organophosphates.

4.4. Effect on the cholinesterase parameter

The results of the present study showed inhibition in plasma cholinesterase or BuChE. The decrease in the activity was in both oral and dermal treatments. The level of inhibition was highly significant. This is because the tested formulation is content of chlorpyrifos which is organophosphate compound (OP) and these compounds are known to inhibit cholinesterases ChE, so the inhibition of this enzyme is the admitted result after the treatment with (OPs). These results are in agreement with **Bushnell *et al.*, (1994)**. reported that a daily subcutaneous injection of chlorpyrifos caused inhibition by 60% to 90% in ChE activity in whole blood of rats and the inhibition prolonged after stopped the doses. In addition, Plasma BuChE is more sensitive to OP exposure than RBC AChE **(Amitai *et al.*, 1998)**; therefore, BuChE is commonly used to monitor occupational exposure to OP compounds **(Khan *et al.*, 2008).**

5. Conclusion

The present study concluded that, the pesticides mixture induced neurotoxicity, genotoxicity, severe histological changes. Also resulted in lipid peroxidation, inhibition in the activities of antioxidant enzymes (Cat) and reduced the glutathione contents of male rats. In addition the results showed the dermal treatments were more toxic and hazard than oral treatments, so caution should be exercised in their handling as prolonged exposure may lead to adverse health effects.

Since mixture of pesticides are widely used in recent years around the world and also become widely used in Egypt, pesticide mixtures continue to pose major challenges for international agencies and their included the data gaps that exist for many individual chemicals, and poorly understood pathways for chemical interaction, these mixtures when present together may induce bio-activation sites resulting in the increase of the observed toxicity of these pesticides mixtures compared with the toxicity of the individual alone. Finally, the results strongly impose the need to more detailed testing of the toxicity of mixture exposure than to one individual.

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