INDIVIDUAL AND MIXTURE EFFECT OF DELTAMETHRIN AND DIMETHOATE ON LIVER: A BIOCHEMICAL, HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL, AND GENOTOXIC STUDY

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ABSTRACT

Many pesticides are used together or in a method, eventually resulting in multiple exposures. These mixtures can lead to unpredicted adverse health consequences in the exposed population. Numerous studies on individual risk assessments are available, but combined usage's toxicity is still to estimate. So, the current study investigated hepatotoxicity induced by exposure to deltamethrin (DLT) and dimethoate (DM) and their mixture in adult male albino rats. **Methods:** Forty adult male albino rats were randomized into five equal groups (n=8); Group I: control, Group II: the vehicle group received 1ml corn oil. Group III: received DLT (5 mg/kg b.w.), Group IV: received DM (20 mg/kg b.w.), Group V: received DLT (5 mg/kg b.w) and DM (20 mg/kg b.w). Treatments were orally gavaged once-daily dose for twelve weeks. **Results:** Separate DLT and DM exposure significantly induced an increase in serum liver enzymes, including aspartate aminotransferase, alanine aminotransferase, alkaline transferase, and lactate dehydrogenase, bilirubin, and liver malondialdehyde levels accompanied by a decrease in serum total protein, plasma cholinesterase enzyme, liver superoxide dismutase, catalase, and reduced glutathione levels. These biochemical alterations were supported by the lesions observed in histological sections, decreasing the expression of Bcl-2 and DNA damage and genotoxicity detected by the comet assay. **Conclusion:** Co-administration of DLT and DM aggravated hepatic dysfunction, exhausting the endogenous antioxidant status and down-regulating the expression of Bcl-2. Thus, these mixtures can increase the harmful effects of each compound on the liver.

Keywords: Organophosphorus; Pyrethroids; liver; Comet assay; apoptosis; oxidative stress

INTRODUCTION

Pesticides are essentially used chemicals in agriculture. They resulted in infinite economic evolution by controlling and eradicating pests and disease vectors. Using pesticides has helped in the provision of food for the growing world population after the World Wars. Following this global change, pesticides became an integral part of world agribusiness. Consequen*tly*, farmers competed for maximum production through the misuse of these substances (**Samih and Ahami, 2019**). However, pesticide usage intensification carries prompt risks to human health during their gradual environmental buildup and accumulation inside human tissues **(Nassar et al., 2020)**.

Environmental contamination with pesticides represents one of the major problems locally and globally. These toxic chemicals were found in air, water, house dust, and even in the tissues of

occupationally and non-occupationally exposed people, especially the adipose tissue, blood, and urine (**Van den Berg et al., 2020)**. According to the World Health Organization, pesticide intoxication presents a major public health problem; about 3 million cases of pesticide poisonings occur per year, killing nearly 250-370,000 people (**Varol et al., 2016)**.

Deltamethrin (DLT) is a synthetic type II pyrethroid, used as an insecticidal and anti-parasitic agent. These pesticides have many advantages, such as being photo-stable, highly efficient at lower concentrations, and easily disintegrated with lower toxicity to other animals' species. However, pyrethroids hazardously affect human health through inhalation, dermal contact, and oral via food and water contamination. Numerous studies have shown that pyrethroids caused liver, reproduction, kidney, and nervous dysfunctions (**Saoudi et al., 2017)**.

One of the most commonly used organophosphate pesticides, DM, is used against a wide scale of insects and mites as well as house flies control. The immense use of DM has led to health hazards to mammals and humans as it can persist in soil and crops. Previous reports have demonstrated that exposure to DM induced endogenous antioxidant/oxidants imbalance, cytotoxicity of various body organs, and lipid peroxidation of the liver in mice and rats **(Zakzook et al., 2020)**.

The increased resistance of pests to particular pesticides leads to the need for combining two or more pesticides. Each active constituent in such formulation possesses a definite mechanism of action against pests, with potentially adverse effects on the exposed non-target animals and humans. Exposure to combined formulations is considered the most common, so it needs more focus regarding exposure assessment, hazard identification, risk assessment, and risk characterization of mixtures rather than single chemicals (**Ramon-Yusuf et al., 2017)**.

The marketing of mixtures of pyrethroids and organophosphorus pesticides (OP) grows to be common in developing countries and has increased toxicity prevalence **(Wang et al., 2020)**. Trials to predict toxicity related to mixtures depending on individual chemicals' acquaintance frequently lead to incorrect and insufficient conclusions. The interactive action due to merging two or more pesticides is not always foreseeable because this mixing may have synergetic, summative, potentiating, or inhibitory, or both effects **(Ramon-Yusuf et al., 2017).** Hence, this work assessed the toxicity of deltamethrin (DLT) and dimethoate (DM) individually and in-combination on the liver.

MATERIALS & METHODS

Chemicals:

Deltamethrin (DLT): Deltamethrin: (99% purity, CAS number: 52918-63-5) and **Dimethoate (DM)**: Dimethoate: (99.9% purity, CA Snumber1219794-81-6) were purchased from Sigma Egypt**. Corn oil:** (vehicle for DLT and DM**)** obtained from Sekem Company, Cairo.

Animals:

Male albino rats (200± 20g, 8 –10 weeks of age), procured and kept up at the Breading Animal House of Faculty of Medicine, Zagazig University. Animals were allowed to accommodate for two weeks to rule out ill animals, then housed and kept under standardized environmental conditions. They were housed at filtered-top plastic cages, temperature maintained (22±3°C), humidity (50%–60%), artificial illumination (12h light/darkness cycles), adequately ventilated place, free of contamination, and feed with ad libitum access to water. The animal accommodation, procedures, and principles for laboratory animal care followed the recommendations of the National Institutes of Health Guide for Handling of Experimental Animal **(Institute of Laboratory Animal Resources, 2011).** The study was

conducted according to the animal care guidelines of the National Institutes of Health (NIH) and the ethics of the Zagazig University committee for experimental animal care and use.

Forty animals were randomly allocated to five groups, with eight rats in each. All the treatment was via oral gavage once daily for 12 weeks; Group I (control): To evaluate the normal variables; Group II (vehicle): received 1 mL corn oil; Group III (DLT group): Each rat gavaged with DLT (5mg/kg body weight). The selected dose 5 mg/kg b.w./day was according to **(Khalatbary et al., 2017)**; Group IV (DM-treated group): Each rat gavaged with DM (20mg/kg b.w.). The selection was according to **(Kamath et al., 2008; Saafi et al., 2011);** Group V (DLT and DM mixture-treated group): Each rat orally gavaged with DLT and DM (the same previously mentioned dose and duration).

At the end of the study, all rats were anesthetized to lessen pain and distress, and a blood sample was withdrawn from the retro-orbital venous plexus by using capillary glass tubes as demonstrated by **Nemzek et al. (2001)** and used for estimating liver function tests, plasma cholinesterase enzyme (PChE). The liver was obtained; part was used as liver homogenate to assess tissue lipid peroxidation, antioxidant enzymes and evaluate genotoxic and DNA breaks using a Comet assay. The other part of the liver was subjected to histopathological investigations and immunohistochemical staining.

Methods

Biochemical investigations:

- Liver enzymes: Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline transferase (ALP) were measured spectrophotometrically as described by **Reitman and Frankel (1957**) and **Young et al. (1975)**, respectively. Serum lactate dehydrogenase (LDH) was measured as described by **Babson and Babson (1973).** The values were expressed as U/L**.**

-Total protein and bilirubin: The methods of **Lowry et al. (1951) and Schmidt and Eisenburg (1975)** were used for measuring the serum total protein and bilirubin consequently. The values were expressed in mg/dl.

-Plasma Cholinesterase (PChE): Estimation of PChE by enzymatic colorimetric assay was performed according to **(Ellman, 1961).** The amount of 2-nitro-5-mercaptobenzoate production correlating with the PChE activity is estimated photometrically. The measurement has been performed by calculating the rise in absorbance at 412 nm.

Tissue lipid peroxidation and antioxidants: Malondialdehyde (MDA) levels in the hepatic tissue were measured according to the method of **Mihara and Uchiyama (1978)**. Antioxidant enzymes were estimated; superoxide dismutase (SOD) as described by **Nishikimi et al. (1972)**, catalase (CAT) following the method of **Aebi (1984)**, reduced glutathione (GSH) as described by **Beutler et al. (1963),** and total antioxidant capacity (TAC) was done consistently with **Koracevic et al. (2001)**.

Alkaline single-cell gel electrophoresis (SCGE**):** Samples from liver tissue were used for assessing the degree of deoxyribonucleic acid (DNA) break by comet assay as described by **Singh et al. (1988).**

Histopathological examination:

Specimens from the liver were dissected, fixed within 10% formalin saline solution for a period greater than twelve hours, dehydrated, then embedded in paraffin wax, serial cut sections of 5-µm-thickness, then fixation on slides and stained with hematoxylin and eosin (H&E) stain (**Bancroft and Gamble, 2007).**

Immunohistochemical and immunohisto-morphometric Analysis:

Immunohistochemical reactions were performed on sections of liver Bcl-2

proteins using a streptavidin-biotin peroxidase system (Biogenex, San Ramon, CA, USA). In brief, liver sections were subjected to dewaxing in xylene and dehydration. Blocking endogenous peroxidase activities performed with 1% H_2O_2 exposure for 15 min. Then, incubated in polyclonal antibodies for Bcl-2 (Santa Cruz, CA, the USA at a dilution of 1: 40). for 30 min at 37 °C. After staining with Diaminobenzidine, sections were hydrated and examined with light microscopy. Cytoplasmic staining for Bcl-2 was considered positive (**Shalaby, 2007)**.

Sections stained with Bcl-2 immunoreaction were subjected to morphometric image analysis by Leica Qwin500 Image Analyzer Computer System (England). The number of cells with Bcl-2-positive immunoreaction for each specimen was calculated in 8 fields then randomly selected and analyzed.

Statistical Analysis:

Results were expressed as mean± standard deviation and analyzed using the Social Sciences Statistical Package (SPSS) (version 20). One-way Analysis of variance (ANOVA) was performed to study the effect of DLT and DM administration on the considered variables. The LSD test was used for multiple comparisons between different groups. Data were considered significant at p-value **<0.05**, and highly significant at p-value **<0.01** and **<0.001**.

RESULTS

No significant difference (P>0.05) between both control and vehicle groups (I &II) was observed regarding the mean values of all measured parameters, so group (I) was used for comparison with other study groups.

Biochemical results: (Table-1)

-Serum liver enzymes: The mean values of liver enzymes AST, ALT, ALP, and LDH levels in the DLT group (III) and DM group (IV) were significantly elevated (P>0.05) compared to the control group (I). The mixture treatment group (V) (DLT+DM) exhibited a significantly high increase (P<0.01) in relation to other groups (I, III&IV).

-Serum total protein and total bilirubin: There were significant decreases (P>0.05) of mean values of serum total protein levels and increased serum total bilirubin levels in the DLT group (III) and DM group (IV) compared to group I. Also, the concomitant exposed group (V) (DLT+DM) revealed significantly (P<0.01) decreased total protein and an increase in total bilirubin levels compared with other groups (I, III&VI).

-Plasma cholinesterase enzyme (PChE): The mean values of PChE (U/L) of DLT treated animals and DM group (IV) were significantly reduced (P<0.001) in contrast to group (I). DM treated group (IV) exhibited a significant decrease compared with the DLT group (III). While rats of group (V) (DLT+DM) presented with significantly reduced levels (P<0.001) compared to groups (I, III&IV).

Hepatic lipid peroxidation and antioxidants:

The mean values of MDA concentrations were significantly raised (p>0.05) in liver tissue of individually DLT or DM-treated animals, whereas no significant changes were detected in group I. However, animals of co-treatment with DLT plus DM displayed significantly elevated (P<0.01) MDA concentrations higher than groups (I, III& IV) (Table-2). While the mean values of GSH, SOD, CAT, and TAC concentrations were significantly decreased (p>0.05) in rats received DLT or DM separately compared to the control group. Whereas, a high significantly (P<0.01) decline of antioxidant enzymes was detected in the mixed treatment group (V), compared to groups (I, III& IV) (Table-2).

Comet assay:

Results of the Comet assay of most hepatic cells in the group I displayed a complete, spherical head, and the comet

tail was minimal (Fig.1). Single DLT or DM administration induced significant raise (P<0.001) in the mean values of unit tail moment level compared with control group values. While combined DLT+DM treated animals revealed significantly increased (P<0.001) tail intensity compared to other groups (I & II& III&IV) (Table-3).

Histopathology:

The light microscopy assessment of hematoxylin and eosin (H&E) stained liver sections from (group I) displayed regular structural lobules with hepatic cells arranged in cords from the central vein to the peripheral, including portal tracts. Liver cells had polyhedral shape and eosinophilic cytoplasm with vesicular nuclei, where narrow sinusoids separated them. The portal areas present in-between lobules (Fig. 2-a).

DLT treatment-induced hepatocyte cord disarranged and dissociation. Some cells displayed several cytoplasmic vacuoles (vacuolar degeneration), and several cells appeared to shrivel with darkly stained pyknotic nuclei. The other hepatocytes still showed preserved structure; they appeared polyhedral with acidophilic cytoplasm with a rounded vesicular nucleus and prominent nucleoli. The central veins were mildly dilated with areas of sinusoidal dilatation and few inflammatory cells (Fig 2-b). However, the liver of the DM-treated group (group IV) showed degenerated hepatocytes with marked cytoplasmic vacuolation (hydropic changes), and many hepatocytes appeared with shrunken darkly stained pyknotic nuclei. Few hepatocytes were polyhedral, having acidophilic cytoplasm and rounded vesicular nucleus with a prominent nucleolus. Areas of sinusoidal congestion and dilatations were present between hepatocytes (Fig 2-c). While combined treated group DLT and DM (group V) revealed disrupted hepatic architecture, central veins were markedly dilated and congested with inflammatory infiltration. Hepatocytes showed widespread hydropic degeneration (cells with marked cytoplasmic vacuolation and swelling). The nuclei were pyknotic in most hepatocytes (Fig. 2-d).

Immunohistochemistry:

Immunohistochemically stained sections from the control (group I) revealed strong positive immunoreactions in most of the hepatocytes for the apoptotic inhibitor marker (Bcl-2) represented by brown granules in the liver tissue. However, the sections from DLT treated (group III) showed moderatelyBcl-2 positive hepatocytes while the DM-treated group showed mildly Bcl-2-positive hepatocytes. In the combined treated group (V), there were few Bcl-2-positive hepatocytes (Fig. 3) and (Table-4).

Table (1): Liver function, total serum protein, bilirubin, and choline esterase in the different groups of the study:

	Control group I	**Vehicle group II**	**Group III (DLT)**	**Group IV (DM)**	**Group V (DLT+DM)**	**p**
AST (U/L)	109.5 ±10.5^{a,b,c}	105.5 ±9.7^{a,b,c}	204.7 ±17.2^{c}	205.4±16.3^{c}	244.7 ±23.3	<0.001*
ALT (U/L)	53.6 ± 4.7^{a,b,c}	50.4 ± 4.8^{a,b,c}	102.3± 11.2^{c}	103.7± 10.5^{c}	122.4± 13.1	<0.001*
ALP (U/L)	107.3± 26.5^{a,b,c}	109.6± 31.1^{a,b,c}	122.1±33.2^{c}	121.4±30.6^{c}	133.1±32.8	<0.001*
LDH (U/L)	39.52±2.35^{a,b,c}	37.44±2.09^{a,b,c}	70.63± 5.70^{c}	71.63± 5.51^{c}	86.93± 4.50	<0.001*
Total serum protein (mg/dL)	7.9 ± 0.52^{a,b,c}	7.7 ± 0.20^{a,b,c}	5.2 ± 0.91^{c}	4.9± 0.85^{c}	3.60± 0.36	<0.001*
Total bilirubin (mg/dL)	1.58 ± 0.02^{a,b,c}	1.49 ± 0.03^{a,b,c}	2.30 ± 0.06^{c}	2.61 ±0.01^{a,c}	3.71 ± 0.05	<0.001*
Choline esterase (U/L)	895.01±85.87^{a,b,c}	889.94±90.54^{a,b,c}	801.52 ± 86.16^{b,c}	579.17±74.42^{c}	421.67±53.89	<0.001*

Values are expressed as mean ± standard deviation (SD) n = 8 animals/group *ANOVA test a: P<0.05, compared with Group III b: P<0.05, compared with Group IV c: P<0.01, compared with Group V

Table (2): Tissue lipid peroxidation and antioxidant enzymes in the different groups of the study:

	Control group I	Vehicle group II	Group III (DLT)	Group IV (DM)	Group V (DLT+DM)	p
MDA (nmol/g)	30.92 ±1.07[a,b]	32.03 ±1.15[a,b]	71.44 ±1.24[b]	70.45 ±1.16[b]	96.34 ±1.32	<0.001*
GSH (mg/g)	56.36± 3.08[a,b]	58.19± 3.47[a,b]	39.45 ± 4.08[b]	40.14 ± 3.09[b]	20.63 ± 3.06	<0.001*
SOD (u/g)	16.91 ± 0.94[a,b]	17.53 ± 0.81[a,b]	7.68 ± 0.46[b]	8.11 ± 0.79[b]	4.65 ± 0.53	<0.001*
CAT (u/g)	1.47 ± 0.08[a,b]	1.53 ± 0.09[a,b]	0.56 ±0.03[b]	0.61 ±0.05[b]	0.13 ±0.06	<0.001*
TAC (mmol/g)	49.38± 1.56[a,b]	47.39± 1.63[a,b]	30.79 ± 0.49[b]	30.47 ± 0.62[b]	16.98 ± 0.51	<0.001*

Values are expressed as mean ± standard deviation (SD) n = 8 animals/group
***ANOVA test a: P<0.05, compared with Groups III&IV b: P<0.01, compared with Group V**

Table (3): Statistical comparison of Comet assay results in the liver of the different groups of the study:

	Control group I	**Vehicle group II**	**Group III (DLT)**	**Group IV (DM)**	**Group V (DLT+DM)**	**p**
Tailed %	5.30 ± 0.25[a,b]	5.24 ± 0.19[a,b]	14.05 ± 0.94[b]	13.83 ± 0.64[b]	21.13 ± 0.91	<0.001*
Tail length (µm)	0.87 ± 0.08[a,b]	0.93 ± 0.07[a,b]	4.76 ± 0.36[b]	4.58 ± 0.23[b]	7.97± 0.46	<0.001*
Tail DNA (%)	0.76 ± 0.07[a,b]	0.82 ± 0.08[a,b]	4.96 ± 0.32[b]	5.14 ± 0.27[b]	8.13 ± 0.64	<0.001*
Unit tail moment	0.67 ± 0.06[a,b]	0.74 ± 0.06[a,b]	18.15 ± 1.05[b]	19.01 ± 1.21[b]	29.02 ± 1.05	<0.001*

Values are expressed as mean ± standard deviation (SD) n = 8 animals/group
***ANOVA test a: P<0.05, compared with Groups III&IV b: P<0.01, compared with Group V**

Table (4): The mean number of Bcl-2 positive cells in the different groups of the study:

	Control group I	**Vehicle group II**	**Group III (DLT)**	**Group IV (DM)**	**GroupV (DLT+DM)**	**p**
Bcl-2 (mean number of cells/field)	9.46 ±2.29[a,b,c]	10.03 ±2.78 [a,b,c]	6.47 ±1.29[b,c]	4.98 ±1.30[a,c]	2.33 ±0.66	<0.001*

Values are expressed as mean ± standard deviation (SD) n = 8 animals/group
***ANOVA test a: P<0.05, compared with Group III b: P<0.05, compared with Group IV**
c: P<0.01, compared with Group V

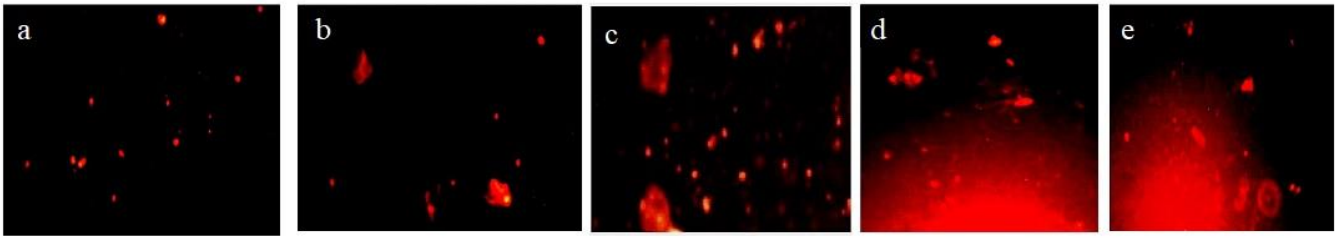

Figure (1): Comet assay figures showing nuclei of liver cells of adult male albino rats; **a-** control group showing almost normal nuclei of condensed type and persevered cells with no damage. **b-** Nuclei of liver cells of the deltamethrin-treated group showed damaged cells with abnormal tailed nuclei. **c-** Nuclei of liver cells of the dimethoate-group showed damaged cells with abnormal tailed nuclei. **d,e-** Nuclei of liver cells of the (deltamethrin +dimethoate) group showed severely damaged cells with abnormal tailed nuclei.

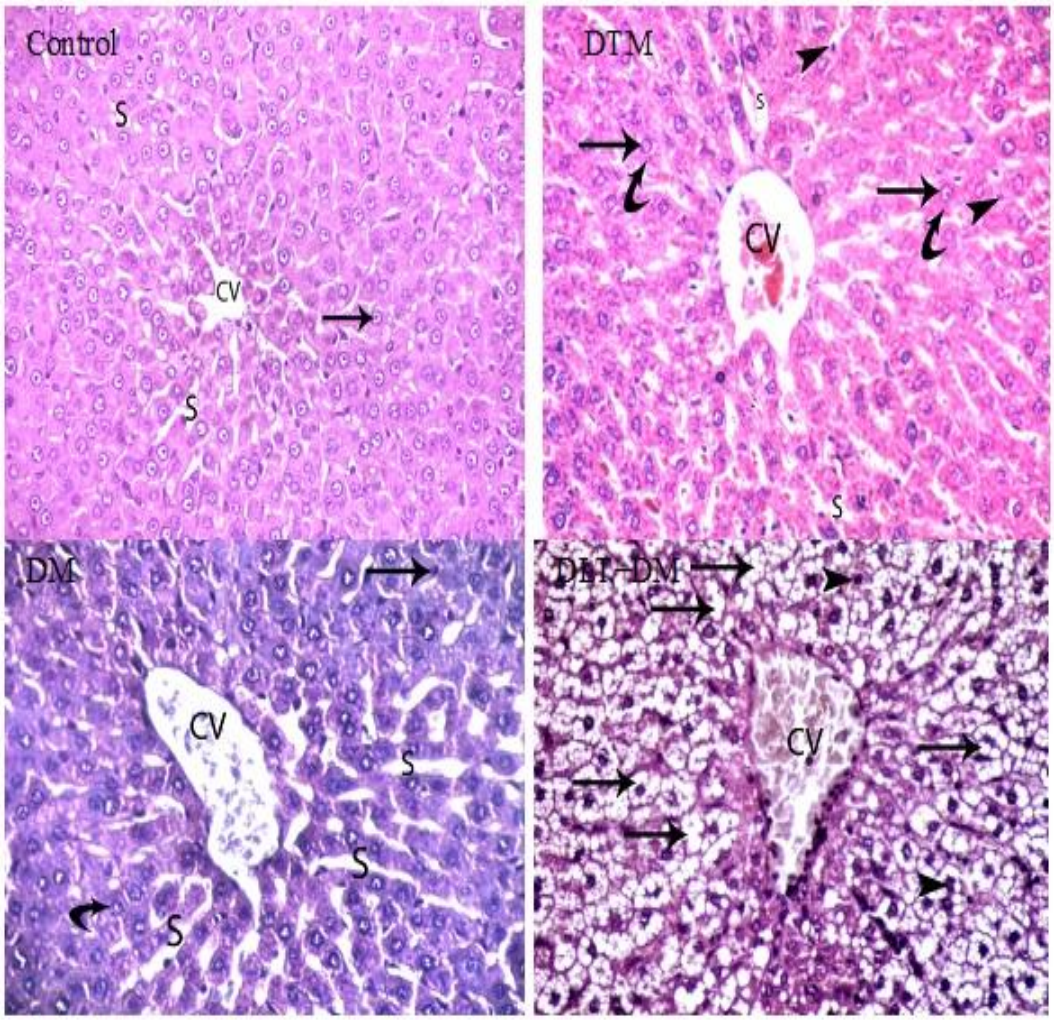

Figure (2): A photomicrograph of a section in the liver from adult male albino rats: **a-** Group I (control): showing normal hepatic architecture with the central vein (CV), hepatocytes radiating in cords and separated with blood sinusoids (s). Polyhedral hepatocytes having acidophilic cytoplasm and rounded vesicular nucleus (arrow) **b**-Group III (DTM) showing mildly dilated central vein (CV). Several hepatocytes have shrunken dark stained nuclei (arrowhead) with areas of sinusoidal dilatation (S) in-between. Some polyhedral hepatocytes with acidophilic cytoplasm are seen (curved arrow) having rounded vesicular nucleus (arrow) **c-** Group IV (DM) showing hepatocytes with cytoplasmic vacuolation and shrunken darkly-stained nuclei (arrow). Few polyhedral hepatocytes with acidophilic cytoplasm and rounded vesicular nucleus are seen (curved arrow). Areas of sinusoidal dilatation (s). **d-** Group V (DLT+DM) The central vein (CV) showed congestion and dilatation. Widespread hepatocytes with vacuolated cytoplasm (arrow). Most of the cells have pyknotic nuclei (arrowhead) (H&Ex400).

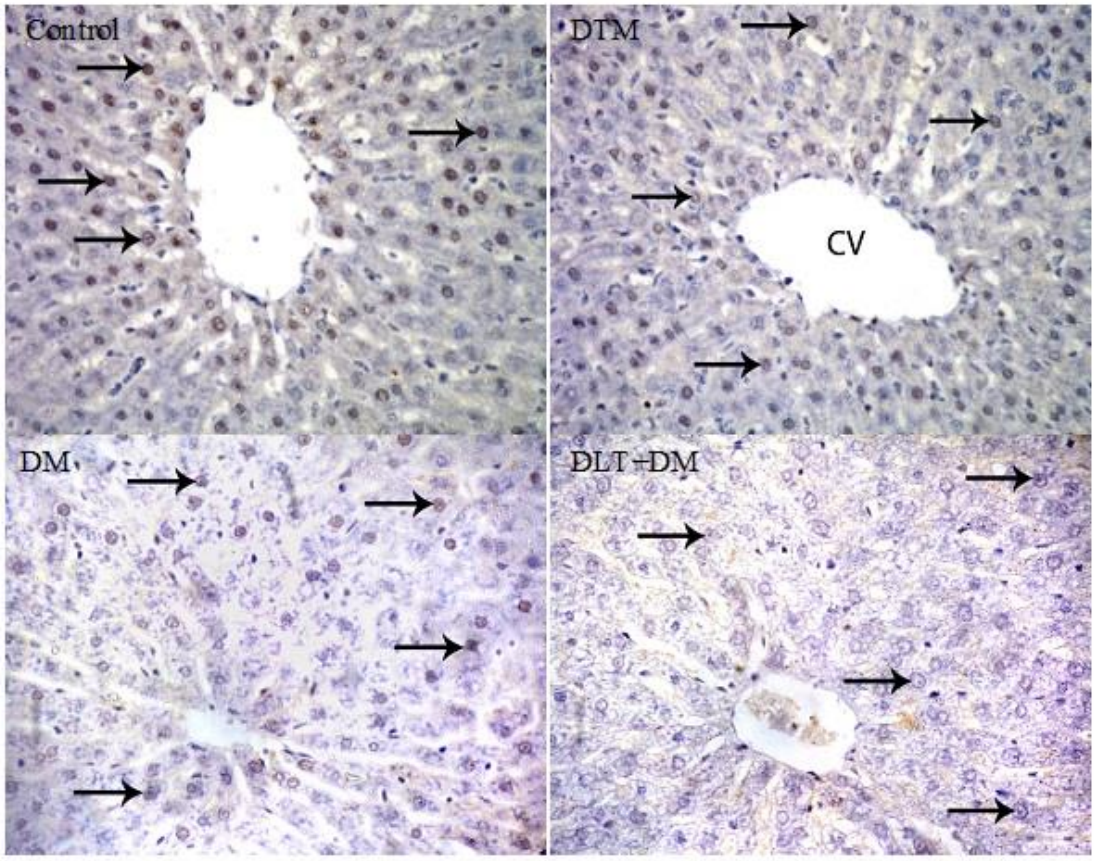

Figure (3): A photomicrograph of a section in the liver from adult male albino rats: **a-** group I (control) showing Bcl-2-positive immunostaining of the hepatocyte nuclei (arrow)**. b-** Group III (DLT) There is a dilated central vein (CV) and hepatocytes show moderately positive Bcl-2 immunostaining in their nuclei (arrow)**. c-** Group IV (DM) showing mild Bcl-2-positive immunostaining in nuclei of hepatocytes (arrow). **d-** Group V **(**DLT+DM) showing weak positive Bcl-2 immunostaining in the nuclei of hepatocytes (arrow) (Bcl-2 X400).

DISCUSSION

The toxicity of pesticides is infrequently restricted to single chemical exposure. People are subjected to various chemicals in their daily life activities. This mixed and combined exposure can result in harmful health effects **(Rani et al., 2020).** The process of hepatic detoxification, metabolism, and biotransformation are key stages in eliminating most of those toxic chemicals. These processes impair liver function, causing hepatotoxicity and serious complications (**Mossa et al., 2013)**. Accordingly, our study evaluated the impacts of separate and mixed oral administration of deltamethrin and dimethoate on adult male albino rats' liver.

Results of liver function revealed a significant increase in serum AST, ALT, ALP, and LDH in the DLT-treated group and DM-treated group. Whereas animals received a mixture of (DLT+DM) showed a highly significant elevation of serum liver enzymes compared to other groups (I & II& III& IV). Similar results were documented by **Chargui et al. (2012).** They detected hepatocellular injury with elevated serum liver enzymes after DLT treatment. The upsurge of hepatic function enzymes owing to DM intoxication in the current study agreed with the study of **Abdel-Daim et al. (2013)**.

Several OP may cause elevated serum liver enzymes; **Mossa et al. (2013)** attributed this finding to hepatocyte injuries, and alteration of membrane

integrity that could be induced by these pesticides, resulting in enzymes leak out hepatocytes. A remarkable increase in these biomarkers' levels could be an indicator of initial hepatocyte injury and dysfunction occurring prior to structural changes of the liver. Moreover, **Kalender et al. (2005)** reported that the levels of hepatic enzymes may fluctuate depending on the duration and dosage of pesticide exposure; this report could explain the marked increase in serum liver enzymes of animals exposed to the mixture of (DLT+DM) in the current study.

The results indicated that individual and combined DLT and DM treatment significantly increased serum bilirubin. In contrast, total protein in the DLT group and DM group was significantly decreased. Also, total protein diminished more with co-treatment of DLT+DM. In parallel with our results, **Abdel-Daim et al.(2013)** stated that DLT induced an increase in total bilirubin along with decreased total protein level. Also, **El-Saad and Elgerbed (2010)** observed the same findings in DM-intoxicated rats. This reduction was accredited by the disturbance of protein metabolism and impaired biosynthetic function of the liver**.** Also, the loss of protein could be either due to reduced synthesis or increased proteolysis activity. Consequently**, Li et al. (2007)** have proposed albumin as a potential biomarker for OP toxicity. All these reports could explain the diminished total protein and albumin metabolism after DM administration in the current study. Another explanation could be due to hepatocyte injury induced by DM, as definite with the up-surge in serum AST and ALT.

Cholinesterase (ChE), or pseudocholinesterase, are formed chiefly within hepatocyte then secreted in circulation. It becomes noticeably inactivated with liver impairment owing to a decline in production. Consequently, any changes in ChE levels reflect the state of hepatocyte function and should be considered an alert biomarker of the diminished synthetic capacity of the hepatocytes and subsequently liver dysfunction (**Brown et al., 1981)**.

The current study demonstrated a significant decrease in plasma ChE level in the DLT group (III) and DM group (IV) although, in co-treatment with (DLT+DM), the degree of PChE inactivation was significantly higher.

The decreased PChE activities of the DLT group in our study agreed with the results of **Yousef et al. (2006)**, who demonstrated that pyrethroids could reduce AChE levels in the RBCs, plasma, and brain. Similar inhibition of plasma AChE was induced by a mixture of OP, diazinon, and DLT **(El-Halwagy and Zaki, 2009)**, and they attributed this inhibition to the combined consequence exerted by OPs and pyrethroids. This inactivated AChE that occurred subsequent to DM and DLT administration could, furthermore, be explained by elevated lipid peroxidation elicited by non-Op pesticides with indirect effects on membrane-bound enzymes as AChE, resulting in reduced activity (**Lo'pez et al., 2007**).

In the present study, a significant rise of liver MDA and decrease of CAT, GSH, and TAC activities were detected in the DLT group (III) and DM group (IV). The mixed (DLT+DM) group showed a highly significant increased liver lipid peroxidation accompanied by decreased antioxidant enzyme levels compared with other groups (I, III&IV).

These findings coincided with **(Chargui et al., 2012)**, who documented that many pesticides could induce oxidative stress revealed by increased MDA and the decrease of SOD levels in exposed humans and animals. DLT could produce oxidative stress, represented by a significant increase in lipid peroxidation, along with the reduction of TAC of DLT-treated rats. This could indicate the role of ROS in DLT-mediated toxicity. Also, DLT-induced lipid peroxidation could induce cell membrane dysfunction.

Therefore, it disturbs the permeability of the hepatocyte cell membrane and antioxidant enzymes. Subsequently, the transfer of ions, various materials, and chemical messengers could be disturbed by membrane receptors. This can cause the diffusion of the liver's antioxidant enzyme into the circulation **(Saoudi et al., 2017).** Moreover, **Sharma et al. (2005)** and **Kamath et al. (2008**) had observed that DM provoked the generation of ROS and hepatic LPO, which was adopted as an explanation underlying OPs-intoxication.

The current study revealed marked DNA damage in examined liver tissue, as represented by significantly elevated tail DNA percentage and intensities of DLT and DM-treated groups. The DNA damage of liver tissue was highly significant in the combined treated group (DLT+DM). These findings were supported by **Madkour (2012**), who proved that Lambda-cyhalothrin, a synthetic type-II pyrethroid, induced DNA damage of the hepatic tissue, as evidenced by DNA fragmentation and severe histological damage. Also**, Ogaly et al. (2015)** reported that DLT caused significant genotoxicity manifested by the widespread tailed nuclei and high tail moment in intoxicated rats.

According to **Ibrahim et al.(2014)**, the mechanism of DLT-induced genotoxicity and DNA damage could be due to either its direct interaction with DNA or by generating ROS, accompanied by nitrous oxide (NO) production, which can restrain cell respiration and initiate apoptotic signals starting DNA injury. Also, **Heikal et al. (2011)** observed that DM administration for 28 days induced DNA injury in the brain tissue assessed by damage index (DI) and damage frequency % (DF) calculated using the comet parameters. Dimethoate-induced DNA damage might be related to lipid peroxidation and the extensive ROS generation, which could lead to fragmentation and breaks in the DNA.

The highly significant DNA damage that was detected in the mixed group V (DLT+DM) agreed with the previous study by **Moore et al. (2010)** concerning the relationship between toxicant-inducing oxidative stress and the extent of DNA damage. They stated that despite differences in their pharmacokinetic properties, most pesticides have at least one characteristic in common: they could induce oxidative stresses, which subsequently results in DNA damage.

In the present study, DLT caused pathological changes; hepatocytes showed signs of vacuolar degeneration or apoptotic characteristics as nuclear pyknosis. Hepatocytes from the DM-treated group displayed marked hydropic and apoptotic changes with sinusoidal congestion and dilatations. While combined DLT and DM exposure induced severe hepatic injury manifested by disrupted hepatic architecture, marked dilation, and congestion of central veins with inflammatory infiltration, also, most hepatocytes showed pyknotic nuclei and hydropic degeneration, diffuse swelling, and hepatocyte hypertrophy.

Near our results, **Khalatbary et al. (2017)** observed cytoplasmic hypereosinophilia, extensive nuclear pyknosis, and loss of intercellular borders of some hepatocytes in sections of the liver obtained from the DLT-treated animals. Also, the results of **Abdel-Daim et al. (2013**) supported our findings that DLT could induce some hepatic injury. The reported histopathological changes in the DM-treated group coincided with **Sharma et al. (2005**), who demonstrated that the liver of DM-exposed rats showed congestion of blood vessels, hemorrhage, cellular infiltrate, vasodilatation, hypertrophy, hydropic and fatty changes.

The results of **Saafi et al. (2011)** confirmed the histopathological findings of our study. They explained that exposure to toxic chemicals could alter the cell's integrity and membrane permeability, resulting in membrane damage or cellular

necrosis with subsequent leakage of liver enzymes into the circulation as a biomarker for hepatic injury. Also, DM and DLT are highly lipophilic substances that could easily bind with cell membranes and facilitate combined damage**.** Moreover, the marked deterioration in liver tissue of the combined pesticides exposed group V (DLT+DM) coincided with **Latuszynska et al. (1999**), who reported that the dermally exposed rats to chlorpyrifos and cypermethrin showed severe structural as well as ultrastructural changes in various organs of treated.

Apoptosis is the key mechanism of many degenerative diseases. It is regulated by the Bcl-2 family proteins and triggered by factors such as toxins. Bcl-2 is an anti-apoptotic family protein that settles in the cytosol and translocates to mitochondria upon induction of apoptosis (**Ali et al., 2017)**. Numerous studies have revealed that exposure to DLT impacted cell survival and induced apoptosis **(Wu et al., 2000)**. Our immunohistochemical study for the apoptotic inhibitor marker (Bcl-2) illustrated that the control group expressed normal strong positive staining of Bcl-2. Conversely, DM, and DLT enhanced apoptosis as both significantly induced low expression of Bcl-2. The liver cells of rats exposed concomitantly to DLT, and DM showed a weak positive immune reaction to Bcl-2.

The decreased anti-apoptotic Bcl-2 immunoexpression in liver cells of the DLT-treated group agreed with the results of **Khalatbary et al. (2017).** They observed significantly increased immunoexpression for apoptotic marker caspase-3 after oral administration of DLT. Our results could be confirmed by **Wu et al. (2000)**, who observed increased P53 and Bax-expression coupled to down-regulated Bcl-2 in brain cortex and hippocampus of DLT-treated rats compared with the control group, resulting in an increased proportion of Bax to Bcl-2, which may explain apoptotic cellular death occurred in the rat brain following DLT exposure. Also, our result was confirmed by over-expressed pro-apoptotic/anti-apoptotic proteins (Bax/Bcl-2) in the testes of rats treated with DM **(Wang et al., 2013)**. These results were near **Ali et al. (2017)**, who reported that immunohistochemical staining of the brain of DLT and DM-treated rats showed increased Bax accompanied by decreased Bcl-2 expression.

Synergism between different types of pyrethroids and OP insecticides has been widely documented. For example, mixtures of cypermethrin/ethion, deltamethrin/triazophos, and deltamethrin/chlorpyrifos showed great synergetic effects in pest control. All these mixtures carry a potential health hazard and risk due to intoxication by both pyrethroids and OP insecticides **(Martin et al., 2003).**

CONCLUSIONS

Our results were the first to illustrate the risk implicated in exposure to such mixtures of pesticides on male albino rats' liver tissue. It could be anticipated that highly significant values regarding the results of all study parameters with the combined administration of DLT with DM were due to an interaction between the two chemicals and pesticide combination can lead to the interactive potentials creating a chemical blend that is further noxious than the adjuvant outcome of each one. Ultimately chronic administration of DLT and DM individually or concomitantly elicited pronounced oxidative stress in the rat liver, which is coupled with marked perturbations in the antioxidant defense system leading to loss of oxidant/antioxidant balance in addition to destructive histopathological changes evidenced by increased liver enzymes, decreased total protein and up-regulating the expression of Bcl-2. Also, the combination of the two pesticides (DLT+DM) caused notably greater damage in the liver than the individual pesticides.

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التأثير الفردى و الخليط للديلتاميثرين والدايميثوات على الكبد: دراسة كيميائية حيوية ,نسيجية ,كيميائية مناعية, وسمية وراثية

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ان استخدام العديد من مبيدات الآفات معًا يؤدى بطريقة أو بأخرى الى التعرض لهذه المبيدات فى شكلها المتعدد مما قد يؤدى في نهاية المطاف إلى عواقب صحية ضارة غير متوقعة على الأشخاص المعرضين. تتوفر العديد من الدراسات حول تقييمات المخاطر الفردية ، ولكن لا يزال تقييم سمية الاستخدام المشترك و الخليط يحتاج الى البحث. لذلك ، تهدف الدراسة الحالية إلى دراسة السمية الكبدية الناتجة عن التعرض لمادة الدلتامثرين والدايميثوات ومزيجهم معا في ذكور الجرذان البيضاء البالغة. تم اختيار أربعين جرذا من ذكور الجرذان البيضاء البالغة و تقسيمهم عشوائيا إلى خمسة مجموعات متساوية (8 جرذان) لكل مجموعة ؛ المجموعة الأولى: مجموعة ضابطة سالبة ، المجموعة الثانية: مجموعة مذيب تم اعطاؤها (1 مل) زيت الذرة. المجموعة الثالثة: تم اعطاؤها الديلتاميثرين (5مجم / كجم من وزن الجسم) ، المجموعة الرابعة: تم اعطاؤها الدايميثوات (20مجم / كجم من وزن الجسم) ، المجموعة الخامسة: تم اعطاؤها الديلتاميثرين (5مجم / كجم من وزن الجسم) والدايميثوات (20مجم / كجم من وزن الجسم). و قد استمر إعطاء العلاجات كلها عن طريق الفم جرعة واحدة يوميا لمدة 12 أسبوعا. تسبب التعرض الفردي للديلتاميثرين والدايميثوات في زيادة ذات دلالة احصائية لأنزيمات الكبد خاصة الأسبارتات أمينوترانسفيراز ، ألانين أمينوترانسفيراز ، الكالاين ترانسفيراز ، و اللاكتات منزوع الهيدروجين ، البيليروبين ، ومستويات المالوندالدهيد فى الكبد مصحوبة بانخفاض في البروتين الكلي في الدم ، إنزيم كولين استريز البلازما ، ديسموتاز الكبد. ، وانخفاض مستويات الجلوتاثيون. و قد صاحب هذه التغيرات الكيميائية الحيوية خلل باثولجى ظهر في الفحوصات النسيجية ، قد ثبت انخفاض فى ال "بى سى إل 2" فى نسيج الكبد وتلف الحمض النووي والسمية الجينية عن طريق استخدام اختبار الكومت . و قد أدي اعطاء الديلتاميثرين والدايميثوات معا إلى تفاقم الخلل الكبدي ، واستنفاذ مضادات الأكسدة الذاتية ، و انخفاض فى ظهور ال "بى سى إل 2" وبالتالي ، فإن هذه المخلطات تؤدى الى تفاقم الآثار الضارة لكل مركب على الكبد.