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Original Research Article

Genotoxic effects of Lambada-cyhalothrin & Dimethoate on Liver of Male Rats

Article History:

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Abstract: ***Background***: The pesticides are one of the most potentially harmful chemicals liberated in the environment in an unplanned manner. Objective: In the present study, we investigated the effect of Lambada-cyhalothrin (LCT) & Dimethoate (DM) on the liver functions of adult male rats. ***Methods***: Forty (40) adult male Wistar albino rats weighing between 180 – 200g were divided into 5 groups of 8 animals each. Two groups were given Lambada-cyhalothrin (20, 40mg/kg) respectively, and Two groups were given Dimethoate (20, 40mg/kg) respectively. The control group was given drinking water. All treated daily by oral gavages for 30 days as one dose/day. ***Results***: There were significant increase ($p<0.05$) in The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels in rats exposed to dimethoate and Lambada-cyhalothrin when compared with control group, which treated with drinking water. In addition, the increase of liver enzyme and the comet tail length revealed a genotoxic potential of Dimethoate & Lambada-cyhalothrin in vivo when compared with control group, which treated with drinking water. The main histological changes on liver tissues in all treated rats with Lambada-cyhalothrin & dimethoate included necrosis of hepatocytes with hypertrophy of portal tract dilated central vein and chronic inflammatory cells infiltrate when compared with control group, marked histopathological changes in liver, intense cytoplasmic immune-expression of caspase 9.

Keywords: Dimethoate, Genotoxicity, Lambada-cyhalothrin, Liver, ALT, AST, ALP, Male Rats.

INTRODUCTION

Insecticide usage is now pervasive on a global scale. Due to their widespread usage in agriculture and pest management, pesticides have been contributing more chemical pollution to the environment (Wang *et al.,* 2011). Consequently, the public and regulatory organizations are becoming concerned about the possible environmental contamination from pesticides (Liu *et al.,* 2010). Pyrethrins I are naturally occurring insecticides that are derived from the dried flowers of Chrysanthemum cinerariaefolium. Over 30% of all insecticides used globally belong to this special class of pesticides (Prasanthi et al., 2005). Their neurotoxicity has been linked to their effects on voltage-sensitive sodium channels, voltage-sensitive calcium channels, voltage-sensitive chloride channels, GABAA receptors, and regulation of neurotransmitter release, including acetylcholine, dopamine, and serotonin (Hossain and Richardson 2011; Ali, 2012). LCT has been discovered to have negative effects on a variety of tissues: it causes oxidative damage and stress-like symptoms in mammalian tissues like the liver, kidneys, and reproductive organs (Righi and Palermo-Neto, 2005; Fetoui *et al.,* 2010; Yousef, 2010).

Acetylcholinesterase is inhibited by organophosphorus (OP) pesticides, which have an impact on the neurological system. The post-ganglionic fibers of the central and peripheral nervous systems, which include acetylcholinemuscarinic receptors, are overstimulated as a result of this inhibition (O'Malley 1997). Acetylcholine (Ach) builds up excessively as a result of this activity, overstimulating cholinergic neurons. If the OP concentration is high, there will be enough mortality in a couple of minutes (Solberg and Belkin 1997).

One of the most significant systemic and contact OP insecticides is dimethoate (DM). (Barski and Zasadowski, 2006) used extensively in agricultural fields on a large number of crops and the domestic environment against a broad range of insects and mites and is also used for indoor control of houseflies.

Generally, the majority of the population is chronically exposed to low doses of DM *via* food, contaminated drinking water, or by application of household insecticides containing DM (WHO/IPCS, 2001). Inhibition of acetylcholinesterase is the main toxic action of DM (Hoffmann and Papendorf, 2006). Additionally, DM induces oxidative stress in humans (Ranjbar *et al.,* 2002) and animals (Sharma *et al.,* 2005a). DM exerts its toxic effects on many tissues and organs including the liver (Saafi *et al.,* 2010).

Our main question is how DM induces subchronic hepatic genotoxicity when administered to rats, with special regard to the role of oxidative stress which can be a leading cause of DNA damage and apoptosis in the liver of treated rats. Oxidative stress is defined as a condition in which pro-oxidant-antioxidant balance in the cell is disturbed; ultimately compromising cell viability (Abdelhady *et al.,* 2017). Also, it may cause cellular apoptosis via both the mitochondria-dependent and mitochondria-independent pathways (Abu Khudir *et al.,* 2019; Krishnendu *et al.,* 2013).

The aim of this study was to evaluate the toxicity of Lambada-cyhalothrin & dimethoate on the liver functions, liver genotoxicity and liver apoptosis by Immunohistochemistry of caspase 9 of adult male rats.

MATERIALS AND METHODS:

2.1. Experimental protocol

The animals were divided into five equal groups, each group consist of 8 rats treated daily by oral gavage for 30 days as follow :

Group1: (control group) was given drinking water.
Group2 & Group3: were given (20,40mg/kg) respectively, of Lambada-cyhalothrin. The LD50 in our study was 612 mg/kg body weight, which has been used previously by other authors (Çelik *et al*., 2003; El-Demerdash, 2007).

Group4 & Group5: were given Dimethoate (20, 40mg/kg) respectively, of Dimethoate. The LD50 in our study was 200 mg/kg body weight, which has been used previously by other authors (Kamath and Rajini, 2007).

2.2. Weights Measurements

Body weight of rats was measured before and after the experiment using a special scale counted for this purpose.

2.3. Blood collection:

After thirty days of treatment, the animals were sacrificed. subsequently, the blood samples were collected by cardiac puncture, 5mL of blood were drawn from each animal of experimental groups, and put in tubes without EDTA, centrifuged at 3000 rpm for 15 minutes, and then serum was separated and kept in the refrigerator at -20ºC until the time of assay.

2.4. Biochemical Tests:

The enzyme activity of AST and ALT was evaluated in the Rats serum following the enzymatic colorimetric method for this purpose a commercial kit (Randox Company) was used and The enzyme ALP was evaluated in Rats serum using a commercial kit produced by Bio Merieux Company.

2.5. Determination of DNA damage by comet assay:

The alkaline comet assay was carried out as described by Olive and Banath (2006) and Speit and Rothfuss (2012) with some modifications. Immediately after euthanasia, 1 g of liver was placed in a Petri dish, add to it 1 ml of cooled cutting solution (NaCl 0.43 gram, EDTA Na 0.8 gram for 100ml distilled water PH 7.5) and cut it well with scissors. Transfer the whole mixture to the Abendrov tube and mix gently with the homogenizer (the tube is placed on ice) 500 rpm Label slide on frosted end using a pencil, not a pen. Pipet 0.1 ml of cells into a 5 ml plastic disposable tube. Add 1.2 ml 1% low-gelling-temperature agarose at 40 °C Mix and rapidly pipet 1.2 ml of cell suspension onto the agarose-covered surface of a pre-coated slide; avoid producing bubbles. The agarose was allowed to set at 40°C for 2 min and the slides were immersed in lysis solution (1.2 M NaCl, 100 mM EDTA, NaOH to pH >13) with freshly added 1% Triton X-100 and 1% DMSO at 4°C overnight. Slides were then placed side by side on the horizontal gel box along with filling the buffer reservoirs with freshly made pH>12.3 electrophoresis buffer containing 0.03 M NaOH and 2 Mm Na EDTA for 25 min before electrophoresis at 0.6 V/cm. Remove slides from electrophoresis chamber and rinse and neutralize in 400 ml of distilled water. Place slides in staining solution containing 2.5 µg/ml of red safe in distilled water for 20 min. Rinse slides with 400 ml distilled water to remove excess stain. The slides were examined by fluorescent microscope.

2.6 Histological Study:

After the collection of blood samples from the animals the following organ liver was isolated In brief the routine sequence of events according to (Suvarna *et al.,* 2018).

2.7 Immunohistochemistry examination of caspase 9

Caspase 9 immunostaining wase done according to the method mentioned by by Alghezi, (2019) with some modifications. Fixed liver tissues was embedded in paraffin wax and the sections were cut into 5 µm thickness. Formalin-fixed slides were deparaffinized in xylene for 5 min, hydrated with 100% ethanol for 1 min then hydrated with 95% ethanol for 1 min, finally they were rinsed in distilled water. Pre-treatments of

tissue sections (Epitope Retrieval) was achieved by heat treatment in an autoclave at 37ºC for 1-2 min in tris buffer then the slides were put in hydrogen peroxide in methanol (0.3%) for 20-30 min; sections were incubated at 37ºC for 10-30 min. Immunoenzyme staining was achieved by rinsing solutions in PBS for 2 min then serum blocking by incubating sections in normal goat serum blocking solution at room temperature for 30 min. These sections were incubated in primary antibody (caspase 9 with dilution 1:100) at 4ºC overnight and were rinsed in PBS for 3-20 min. Peroxidase blocking was carried out by incubating sections in peroxidase blocking solution (3% H2O2 in PBS) for 10 min at room temperature than were rinsed in PBS for 2-3 min. After that, sections were incubated in an anti-rabbit secondary antibody (Dako) for 30 min at room temperature then rinsed in PBS for 3-10 min. Additionally, sections were incubated in DAB peroxidase substrate and rinsed in PBS for 2-3 min. Counterstaining of sections was carried out with hematoxylin solution then washed in running tap water for 2-5 min. Following this step, dehydration was carried out in graded ethanol (95%, 100%) for (1, 2-3) min respectively. Finally, Sections were cleared in xylene for 2-5 min followed by their coverslipping with mounting medium. The slides were ready to examine under a light microscope equipped with a digital camera.

2.8. Statistical analysis:

Statistical analyses were done utilizing the computer data processing (SPSS, version 26). A probability value ($P<0.05$) was considered to be statistically significant. And used to calculate least significant difference (L.S.D.) values for the comparison of means following. According to comet type , the scored comet categorized for five groups depended on the tail DNA percent as described by (Srivastava and Singh, 2020) . comet percent and represented histogram were conducted by Microsoft excel 2010 (Microsoft corporation, USA). The significant difference among different treatment groups were assessed by Chi squire test under P value 0.05 by employing IBM SPSS software v.21 (IBM,USA).

RESULTS:

3.1. Effect of Dimethoate & Lambada-cyhalothrin on Body weight of adult male rats:

The obtained results revealed a significant decrease ($p<0.05$) in body weight of the male rats treated with Lambada-cyhalothrin and Dimethoate at dose (20,40)mg/kg when compared with control group (table 1). while the rats treated with Dimethoate (20,40) mg/kg showed a significant decrease($p<0.05$) in body weight when compared with Lambada-cyhalothrin (20,40) mg/kg (table1).

Table 1 : Effect of Dimethoate & Lambada-cyhalothrin on Body weight of adult male rats.

Treatments mg/kg	Mean ± SD of Rats Weight in gm		p. value
	Before treatment	After 1 month	
Group1	171.3 ±16.5	260.0 ± 22.8^{a}	0.002
Group2	159.8 ± 8.58	239.1 ± 15.6^{b}	0.000
Group3	162.8 ± 15.4	225.0 ± 10.0^{bc}	0.001
Group4	163.5 ± 11.1	210.7 ± 16.9^{cd}	0.008
Group5	172.7 ± 15.3	197.5 ± 2.25^{d}	0.013
p. value	**0.421**	**0.000**	
LSD	**Non-Sig**	**18.0**	

Values are means ± S.E.
Different letters refer to significant differences ($p<0.05$).
Same letters refer to No significant differences ($p<0.05$).

3.2. Effect of Dimethoate & Lambada-cyhalothrin on Liver Enzymes of adult male rats:

The results showed asignificant increase ($p<0.05$) in Liver enzymes ALT, AST and ALP level of the male rats treated with dimethoate & Lambada-cyhalothrin at dose (20,40)mg/kg when compared with control group (table2). while the rats treated with Dimethoate (20,40) mg/kg showed a significant increase($p<0.05$) in Liver enzymes ALT, AST

and ALP level when compared with Lambada-cyhalothrin (20,40) mg/kg (table2).

Table 2 : Effect of Lambada-cyhalothrin & dimethoate on Liver Enzymes of adult male rats.

Treatments mg/kg	Mean ± SD of Liver Function Tests		
	ALT(UL)	AST (UL)	ALP(UL)
Group1	7.16±0.75^{c}	33.1± 2.48^{c}	109.7±1.21^{e}
Group2	10.0± 0.89^{b}	44.5± 2.88^{b}	124.0± 5.54^{c}
Group3	10.5± 1.04^{b}	45.1± 3.31^{b}	121.5± 1.51^{d}
Group4	17.6± 0.81^{a}	56.7± 1.21^{a}	131.5± 1.51^{b}
Group5	18.3±0.81^{a}	57.1± 1.60^{a}	137.0±1.41^{a}
p. value	**0.000**	**0.000**	**0.000**
LSD	**1.03**	**3.31**	**2.88**

Values are means ± S.E.
Different letters refer to significant differences ($p<0.05$).
Same letters refer to No significant differences ($p<0.05$).

3.3 Effect of Lambada-cyhalothrin & dimethoate on DNA damage of Liver of of adult male rats.
Classification of comets based on tail DNA%. Categories of comet: (**A**) undamaged cells (tail DNA% <5); (**B**) low damaged cells (tail DNA% 5–25); (**C**) moderately damaged cells (tail DNA% 26–45); (**D**) highly damaged cells (tail DNA% 46–80); (**E**) extremely damaged cells (tail DNA% >80).

		undamaged nomber		low damaged cells		moderately damaged cells		highly damaged cells		extremely damaged cells	
groups	total scored cells number	number	%	number	%	number	%	number	%	number	%
Group1	321	198	61.68 %	84	26.17 %	33	10.28 %	4	1.25%	2	0.62 %
Group2	295	34	11.53 %	72	24.41 %	102	34.58 %	62	21.02 %	25	8.47 %
Group3	321	11	3.43%	27	8.41%	61	19.00 %	117	36.45 %	105	32.71%
Group4	319	7	2.19%	24	7.52%	47	14.73 %	140	43.89 %	101	31.66%
Group5	325	3	0.92%	21	6.46%	42	12.92 %	143	44.00 %	116	35.69%

Table (3) number and percent of comet types in different treatment groups

Table (4) statistical analysis compression among different treatment groups

		Chi-square	DF	P value
Group1	Group 2	221.9	4	0.000
	Group 3	409	4	0.000
	Group4	437	4	0.000
	Group 5	469.6	4	0.000

Group 2	Group 3	107.7	4	0.000
Group 4	Group 5	3.09	4	0.54
Group 2	Group 4	137.3	4	0.000
Group 3	Group 5	11.9	4	0.01

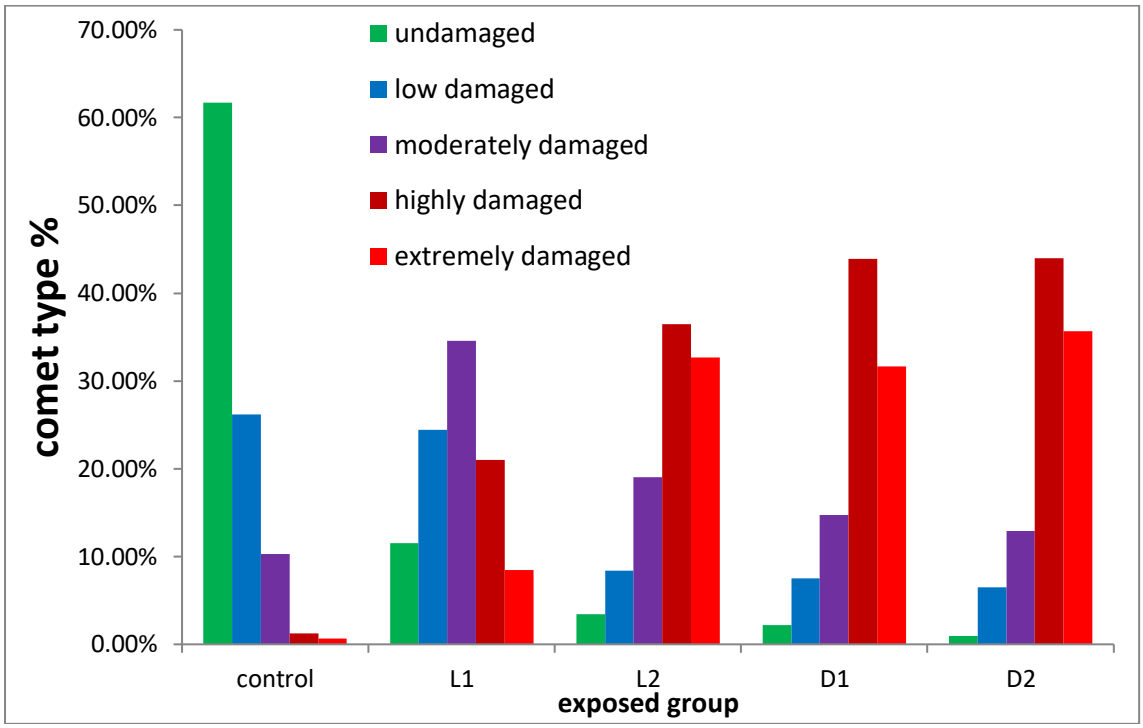
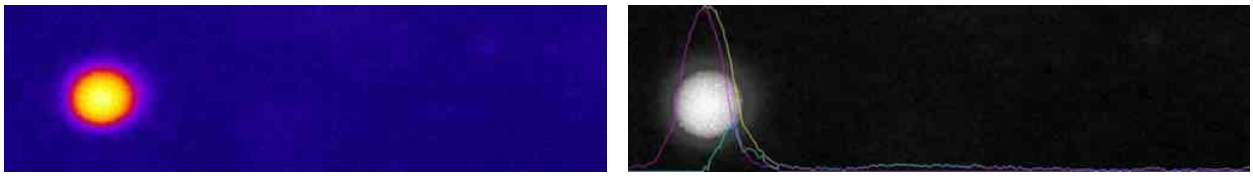
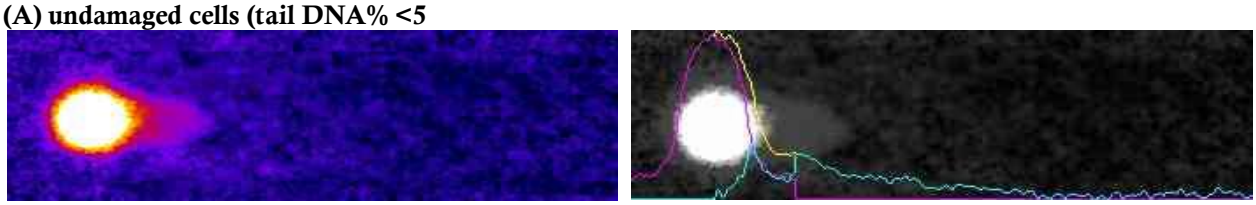


Figure (1) the distribution of comet types for each treatment group. Control group, (L1) LCT- treated group (20mg/kg), (L2) LCT- treated group (40mg/kg) (D1) DM - treated group (20mg/kg) (D2) DM - treated group(40mg/kg).

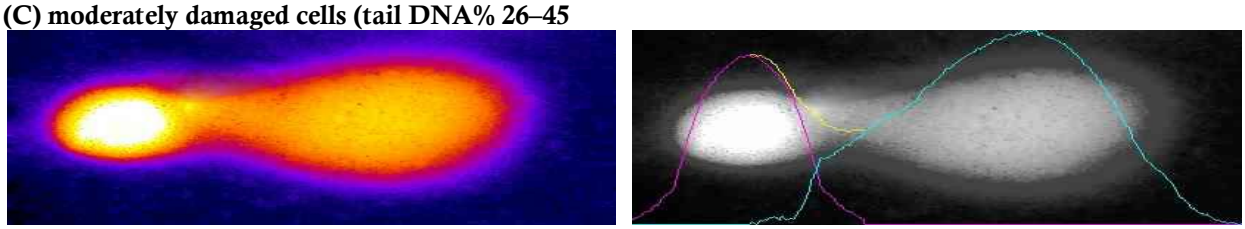


(A) undamaged cells (tail DNA% <5

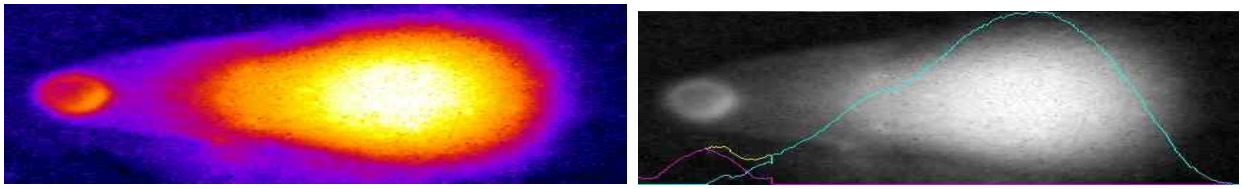


(B) low damaged cells (tail DNA% 5–25

(C) moderately damaged cells (tail DNA% 26–45



(D)highly damaged cells (tail DNA% 46–80



(E) extremely damaged cells (tail DNA% >80).

Figure (2)A-E represent comet categories and analysis by comet score software , the left represent the actual comet , the right represent the analyzed comet (red trace for head DNA intensity , cyan trace for tail DNA intensity and yellow trace for overall DNA intensity)

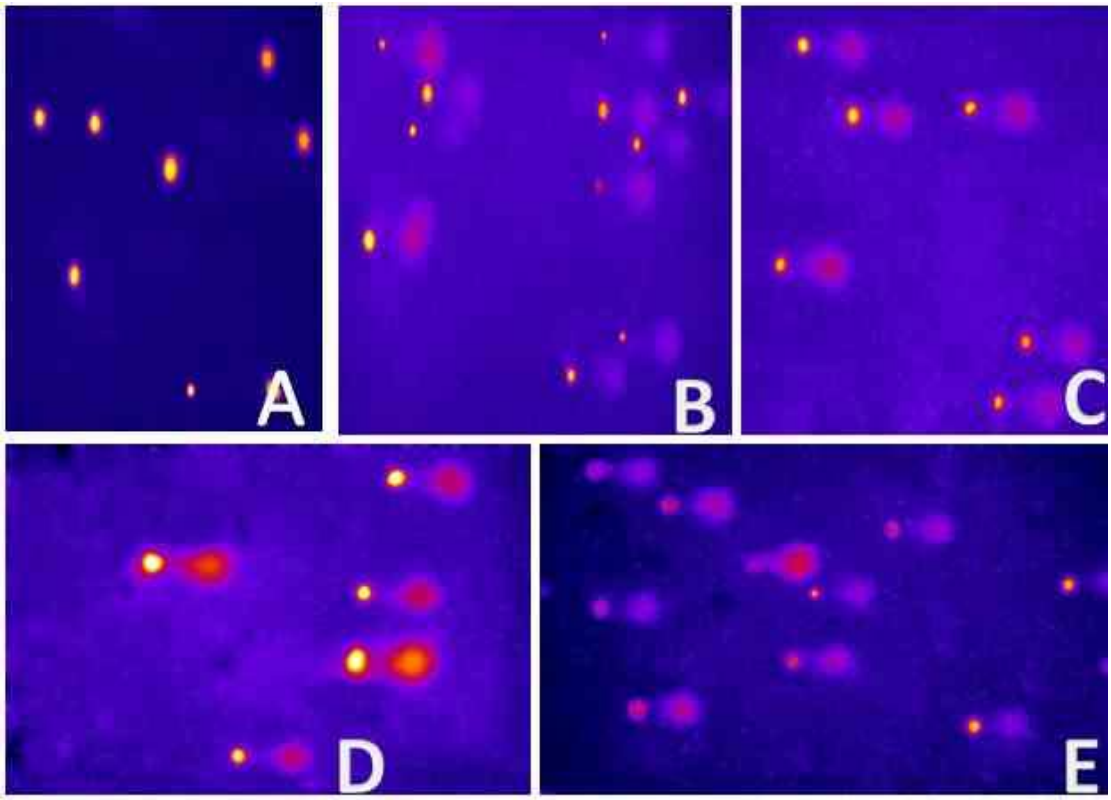


Figure (3): Photomicrographs representative the different degrees of DNA damage in the liver of male rats as evaluated by the comet assay after treatment by Lambada-cyhalothrin & dimethoate. (A) Control group, (B) LCT- treated group (20mg/kg), (C) LCT- treated group (40mg/kg) (D) DM - treated group (20mg/kg) (E) DM - treated group(40mg/kg).

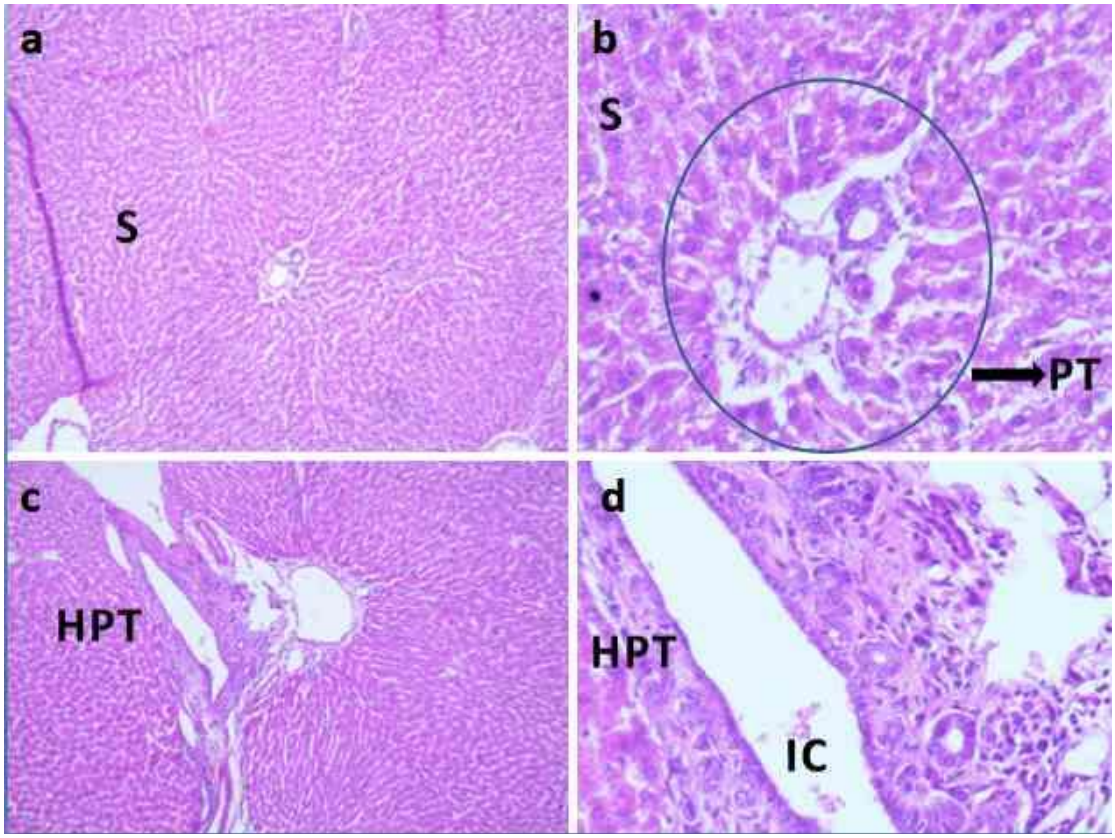
2.4. Effect of Lambada-cyhalothrin & dimethoate on Liver tissue of adult male rats.

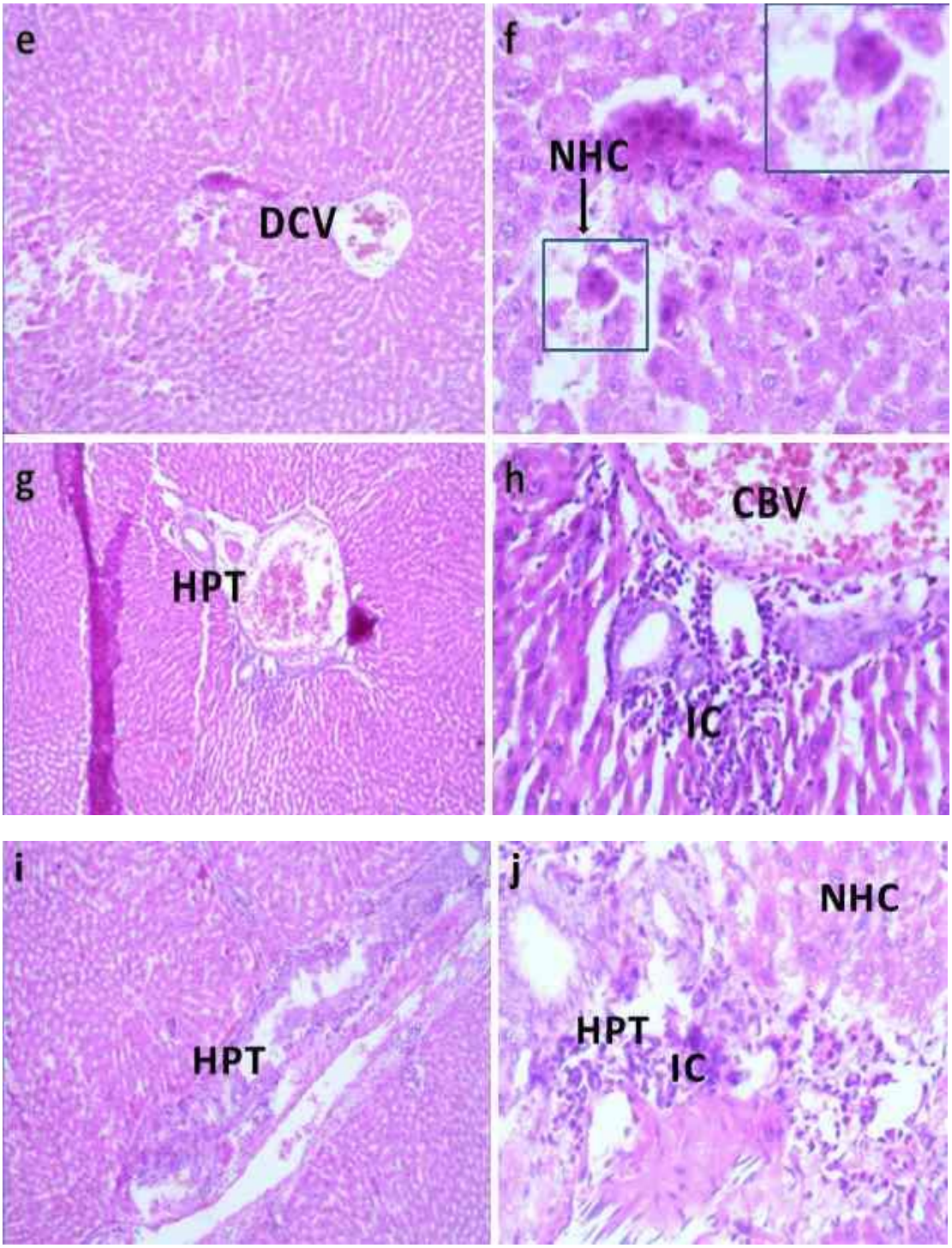
The main histological changes on liver tissues in all treated rats with Lambada-cyhalothrin & dimethoate included necrosis of hepatocytes with hypertrophy of portal tract dilated central vein and chronic inflammatory cells infiltrate (table5).

Table 5 : Effect of Lambada-cyhalothrin & dimethoate on Liver tissue samples of adult male rats.

Liver	Group1 (Control)	Group2 (L1)	Group3 (L2)	Group4 (D1)	Group5 (D2)	Total
	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %
Normal	6 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (20.0)
Mild	0 (0.0)	4 (13.3)	1 (3.3)	2 (6.7)	2 (6.7)	9 (30.0)

Moderate	0 (0.0)	2 (6.7)	4 (13.3)	4 (13.3)	4 (13.3)	14 (46.7)
Sever	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	1 (3.3)
Total	6 (20.0)	6 (20.0)	6 (20.0)	6 (20.0)	6 (20.0)	30 (100%)
CalX^2 = 37.46 TabX^2 = 21.03 DF= 12 P. value < 0.001 Significant						



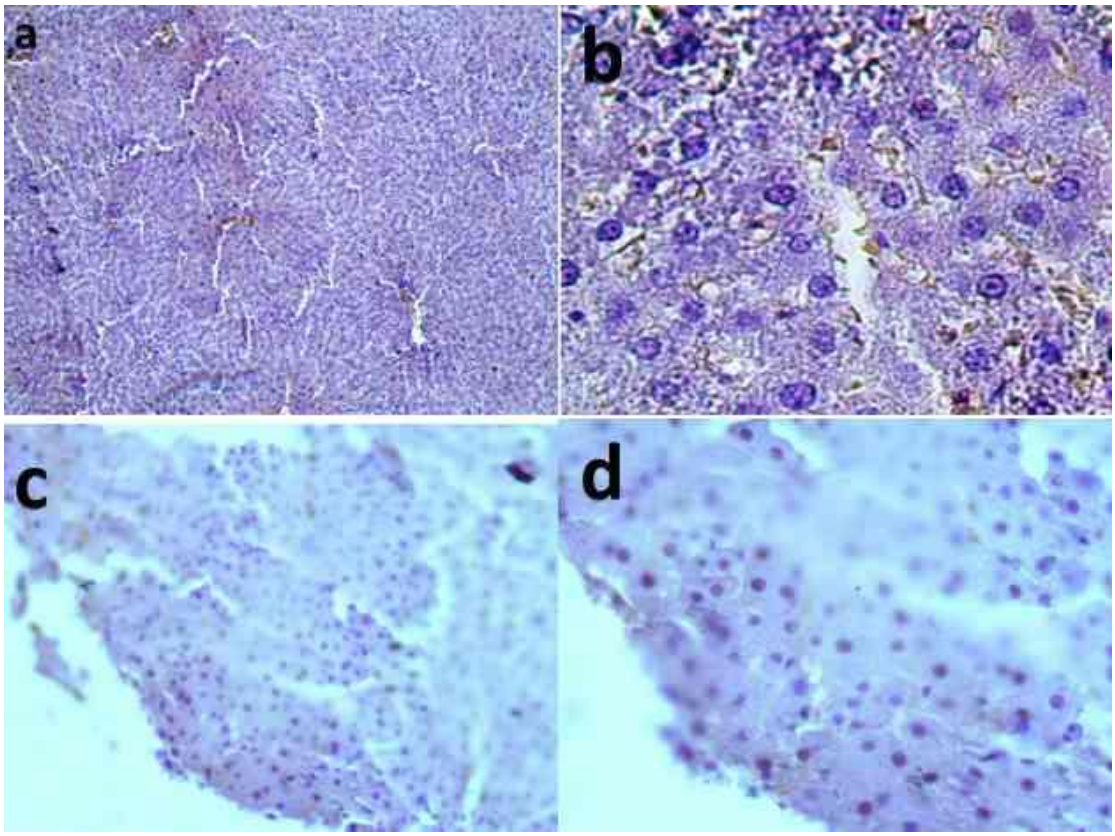


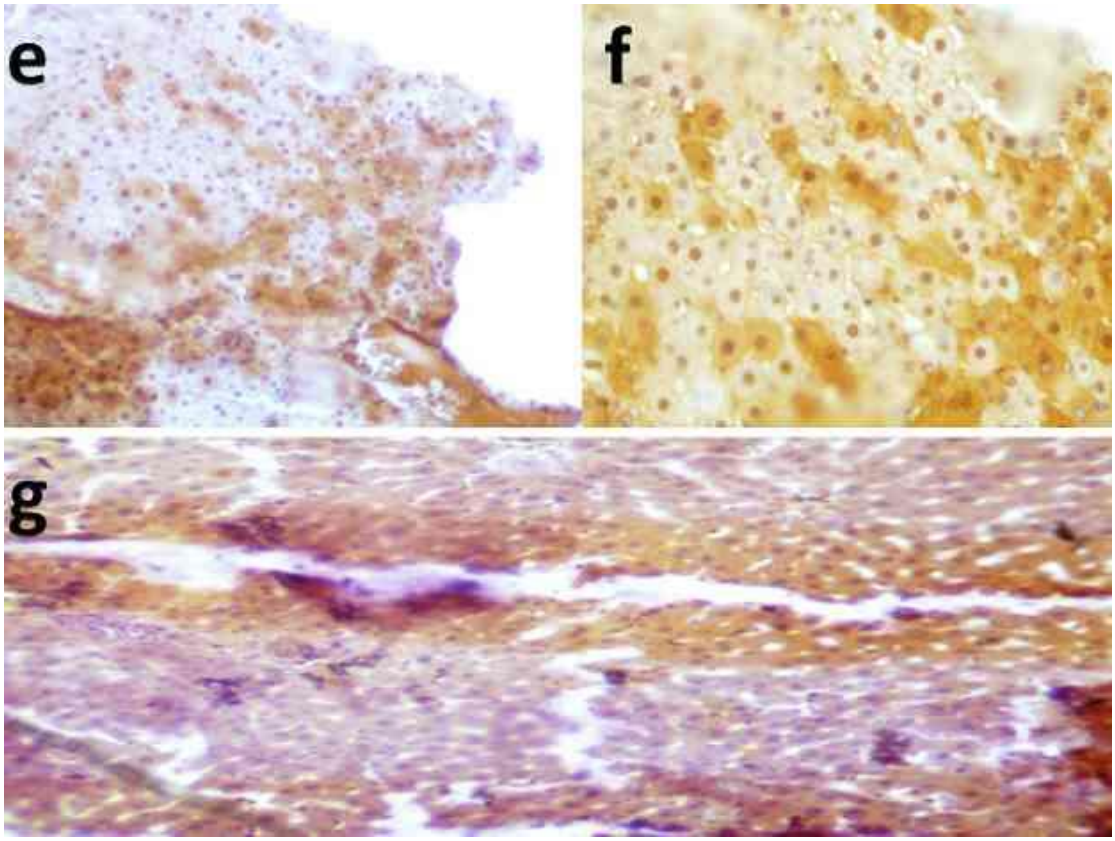
Fig(4) Light micrographs of rat liver tissue stained by hematoxylin-eosin (H&E) in control and treated groups. The control reveals a normal liver hepatocyte and a normal portal tract (a, b) (×10,×40 respectively). Rats treated with lower dose (LCT) show hypertrophy of portal tract with chronic inflammatory cells infiltrate(c, d) (×10,×40 respectively). Rats treated with higher dose (LCT) show dilated central vein, necrotic cells around portal tract and inflammatory cells infiltrate (e, f) (×10,×40 respectively). Rats treated with lower dose (DM) show hypertrophy of portal tract with congested blood vessels and inflammatory cells infiltrate (g, h) (×10,×40 respectively). Rats treated with higher dose (DM) show necrotic hepatocytes, portal tract hypertrophy and inflammatory cells infiltrate (i, j) (×10,×40 respectively).

S sinusoids, PT portal tract, HPT Hypertrophy of portal tract, IC inflammatory cells, DCV dilated central vein, NHC necrotic hepatocytes, CBV congested blood vessels.

Table 6 : Effect of Lambada-cyhalothrin & dimethoate on Immunohistochemical of caspase 9 in the Liver tissue of adult male rats.

Liver	Control	L1	L2	D1	D2	Total
	No. & %	No. & %	No. & %	No. & %	No. & %	No. & %
Normal	6 (20.0)	2 (0.0)	2 (0.0)	3 (0.0)	1 (0.0)	14 (46.7)
Mild	0 (0.0)	4 (13.3)	2 (3.3)	1 (6.7)	2 (6.7)	9 (30.0)
Moderate	0 (0.0)	0 (6.7)	2 (13.3)	1 (13.3)	3 (13.3)	6 (20.0)
Sever	0 (0.0)	0 (0.0)	0 (3.3)	1 (0.0)	0 (0.0)	1 (3.3)
Total	6 (20.0)	6 (20.0)	6 (20.0)	6 (20.0)	6 (20.0)	30 (100%)
CalX^2 = 19.8TabX^2=21.03DF= 12P. value 0.070non-Significant						





Figure(5): IHC results of caspase 9 in the liver of control and treated groups. The control negative cytoplasmic caspase 9 staining in liver tissues (a, b) (×10,×40 respectively). Rats treated with lower dose (LCT) show mild cytoplasmic caspase 9 staining in liver tissues (c, d) (×20,×40 respectively). Rats treated with lower dose (DM) show moderate cytoplasmic caspase 9 staining in liver tissues (e, f) (×20,×40 respectively). Rats treated with higher dose (DM) show moderate cytoplasmic caspase 9 staining in liver tissues (g) (×20).

DISCUSSION:

Pollutants, chemicals and inflammation in the body can probably Increases the production of ROS and probably cause a change in the balance of cellular redox level ultimately resulting in more oxidative damaged biomolecules. Altering the natural redox balance can affect the activity of several enzymes and cell signaling pathways in tissues, which can be a key mechanism for causing xenobiotic intoxication and facilitating the pathogenesis of many diseases(Mishra and Srivastava 2015).

In toxicological studies, body, organ and relative organ weights are important criteria for evaluation of organ toxicity (Heikal *et al.,* 2011) .In the present study, oral administration of Dimethoate and LCT resulted in a significant reduction in body weight of rats. The reduction in body weight may be due to the action of Dimethoate and LCT that produced oxidative stress. It was discovered in the current investigation that insecticides cause experimental animals' body weights to decrease (Jayusman *et al.,* 2014; Ogutcu *et al.,* 2006). The proportional body weight loss in the treated groups is a sign of liver toxicity. Only in the animals given the highest dose did that drop become substantial. Increased catabolic processes like glycogenolysis, lipolysis, or proteolysis could be to blame(Belaid-Nouira *et al.,* 2013). The combined effects of cholinergic and oxidative stress, as well as the general accelerated breakdown of lipids and proteins as a direct result of pesticide exposure, may be to blame for this decrease in body weight (Kopjar *et al.,* 2018; Albasher *et al.,* 2019).

The liver is where foreign substances are biotransformed and detoxified, and it is also where chemical attacks like dimethoate poisoning are most likely to occur (AL-Awthan *et al.,* 2012). One of the vital organs that is vital to the process of eliminating toxins from the body and detoxifying is the liver. Hepatic abnormalities may result from routine daily exposure to a variety of exogenous chemicals and pollutants, such as pesticides. One of the most sensitive markers used in the diagnosis of hepatotoxicity is the presence of serum ALT, AST, and ALP (Kutlu *et al.,* 2007 and Saafi *et al.,* 2011). Pesticide exposure damages the liver and causes other body organs and hepatocytes to release cytosolic enzymes into the blood (Dewan *et al.,* 2004 and Ncibi *et al.,* 2008).

Hepatic biomarker blood enzyme values, such as

those for alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), are typically utilized in the evaluation of liver damage caused by dimethoate and pyrethroids (El-Demerdash, 2004; Stockham and Scott, 2002). Alanine's -NH2 group is transferred by the enzyme ALT to a keto acid acceptor. The amino acid alanine is metabolized in part by aspartate transaminase. Large amounts of aspartate (AST) and alanine transaminase (ALT) enzymes are present in hepatocytes, and liver injury causes these enzymes to diffuse into plasma. Hepatocyte cytosol has a large amount of ALT, which is a sign of damaged liver cells (Kalender *et al.,* 2010).

Whether a liver injury has occurred is determined by the enzyme alkaline phosphatase (ALP). The measurement of serum ALP is frequently used to assess liver impairment (Bradberry *et al.,* 2005). According to Tanvir *et al.* (2015), the release of these liver function biomarkers into the blood circulation as a result of organophosphate exposure could be responsible for the observed elevation of these biomarkers' levels and represent changes in the hepatocytes' membrane permeability (Raina *et al.,* 2015). Under the stress of pesticides, a clear increase in liver ALT and AST has been seen in the current experiment. Studies from several fields have provided strong support for this increase in the aforementioned characteristics (Sahni and Saxena, 2001). The rise in transaminase activity in the serum is a sign of cellular leakage and a loss of the functional integrity of the hepatic cell membrane, which allows the enzymes to escape into the sinusoidal gaps and travel to the intralobular vein (Rahman *et al.,* 1996). Insecticides may cause oxidative stress, which produces free radicals that may cause DNA damage. Reactive oxygen species (ROS) and oxidative stress have been linked to pesticide toxicity in a number of studies, and it is generally acknowledged that ROS eventually result in DNA damage (Bertram and Hass 2008; Heikal *et al.,* 2012). The comet test has been extensively employed in the fields of genetic toxicology and environmental biomonitoring as a biomarker for DNA damage. The DNA strand breaks, double strand breaks, adduct forms, DNA-DNA cross-links, and DNA-protein cross-links that the comet assay discovered might all be the cause of the DNA damage (Mitchelmore and Chipman 1998). The alteration of the mitochondrial membrane potential is linked to oxidative stress-mediated hepatocyte injury (MMP). When measuring the activity of the mitochondrial respiratory chain, electron transport networks, and activation of the mitochondrial permeability transition, the MMP, which denotes the energetic state of the mitochondria in a living cell, is frequently used (Ly *et al.,* 2003). Reduced MMP in isolated hepatic cells may point to the hepatic system's functional dysfunction. In the current study, DNA damage was found by comet assay, and this excess ROS production may directly fragment the hepatic DNA.

Our histological results demonstrated that Lambada-cyhalothrin & Dimethoate caused structural changes in the liver tissue of Wistar rats. Rats treated with lower dose (LCT) show hypertrophy of portal tract with chronic inflammatory cells infiltrate. Rats treated with higher dose (LCT) show dilated central vein, necrotic cells around portal tract and inflammatory cells infiltrate. Rats treated with lower dose (DM) show hypertrophy of portal tract with congested blood vessels and inflammatory cells infiltrate. Rats treated with higher dose (DM) show necrotic hepatocytes, portal tract hypertrophy and inflammatory cells. The emergence of mild foci of necrosis were caused by pesticide treatment. Cell degeneration results in necrosis, which is characterized by organelle enlargement and amorphous cytoplasm, followed by nuclei shrinking and dissolving (Campos-Pereira *et al.,* 2012).

Additionally, the presence of inflammatory cells infiltrate in the liver tissue of treated groups indicates inflammation and a heightened sensitivity to the toxicant. These findings concur with those made by Tripathi and Srivastav (2010), Heikal *et al.* (2012), and Elzoghby *et al.* (2014), who demonstrated that OP pesticide treatment causes inflammatory cells infiltration.

Immunohistochemical observations in our study revealed increased nuclear and cytoplasmic expression of caspase 9 in the hepatic tissue of LCT and DM exposed rats compared with the control group suggesting that pesticide is a potent inducer of apoptosis. These results agree with those of the previous studies that reported an increase in caspase3 activity in cardiac tissue of rats after diazinon administration for 4 weeks (Razavi *et al.,* 2013). Furthermore, subchronic exposure to dichlorvos was reported to increase caspase 3 and 9 activities in endometrium tissue (Oral *et al.,* 2006). These findings support the hypothesis that OP, including DM, induces apoptosis by activating caspase 3. (Masoud et al., 2003). According to Güney et al. (2007), That is caused by activated caspase-3 and -9. These findings support the hypothesis that OP, including DM, induces apoptosis by activating caspase 3. (Masoud et al., 2003). According to Güney et al. (2007), various cellular components associated to DNA repair and control during apoptosis are destroyed as a result of active caspase-3 and -9. These results are also influenced by intracellular ROS production and/or cellular antioxidant deficiency (Serbecic and Beutelspacher, 2005). According to our findings, Zhang et al., found that following OP exposure, caspase 9 and 3 activities increased in a concentration-dependent manner (Zhang et al., 2018). Also, Shiri et al. reported that the activities of caspases were

increased in the diazinon-treated group (Shiri *et al.,* 2016). OP-induced apoptosis may be induced either through extrinsic pathways (death receptors) or intrinsic pathways (mitochondria, DNA damage, and/or endoplasmic reticulum stress) and both pathways cause caspases activation (Kankaya and Kaptaner, 2014).

In general, the caspases are crucial for the activation and execution of apoptosis. The main intrinsic pathway is characterized by mitochondrialdysfunction, with the release of cytochrome c, activation of caspase 9, and subsequently of caspase 3 (Porter and Jänicke, 1999). In this relation, our data demonstrate significantly greater activities of caspases 3 and 9 in rats exposed to DM and LCT, which reaffirmed their role in apoptotic pathway. Earlier studies of pesticides and organic pollutants also demonstrated activation of caspases 9 and 3 (Jin *et al.,* 2011; Kijima *et al.,* 2004).

CONCLUSION:

A pervasively-used Lambada-cyhalothrin & Dimethoate helps in minimizing the damages caused by pests, but its exposure could be harmful for animal as well as human population. This study has exposed the consequences of insecticides exposure on liver enzyme. Also, we have found that the administration of doses of Dimethoate & Lambada-cyhalothrin induced DNA damage in liver cells detected by comet test. hepato-histological abnormality and apoptosis in rat liver. Further explorations at the molecular levels are justified to evaluate the full magnitude of the impact of insecticides exposure on human health.

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