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Chronic Impact of Contaminated Grapes with Pesticides Residues on some Biological Activities in Albino Rats

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Abstract

Aim: Pesticide residues contamination in exported grapes has been monitored in this study, then the obtained results of this survey were used to create laboratory simulations of mixtures that would be encountered by a consumption of contaminated grapes. **Experiment:** Malathion, lambada-cyhalothrin and diniconazole were the highest frequented pesticides in grapes. It has been detected in 45 (75%) samples out of 60 grapes samples were analyzed. The impact of those pesticides residues were applied in mixtures typical of what real exposure would experience has been studied. Rats have been treated with two different concentrations the higher and lower concentration which detected in grapes. **Results:** Our results reported that the exposure to pesticide mixture induced dose dependent effect in experimental animals expressed by significant inhibition of AChE, hyperglycemia that it is a mechanism of oxidative stress induced degeneration in liver and pancreas tissues. **Conclusion**: results indicated that low-level, widespread exposures to pesticide mixtures could impair biological activities in rats. It also shows that traditional approaches to assessing the pesticide toxicity effects one pesticide at a time completely miss understand the health impact of pesticides residues exposure. Further studies to examine the molecular changes of pesticides mixtures exposure are recommended.

Keywords: Pesticides residue, Pancrease, Cholinesterase (ChE), Oxidative stress, total thiol protein

Introduction

Monitoring of pesticides residues as food contaminant is an essential element for ensuring the safety of the food supply. Series of measures of good agricultural practices including optimum dosage, number of application and maximum intervals between application and harvest can be used to keep residue levels as low as possible. Implementation of these measures ensures that the applied pesticides are safe as possible (Tilman et al.,2002). Fruit and vegetables sold in supermarkets contain more than one type of pesticide residue. Six organochlorine and three pyrethroid pesticides were determined in grape, orange, tomato, carrot and green mustard based on solvent extraction followed by solid phase extraction (**Sharif** et al., 2006). Very little research has conducted on the safety of these pesticide mixtures, but some studies have highlighted potential risks to the immune system or behavioral changes. Various human health related concerns are associated with pesticides, ranging from short-term impacts such as headaches

and nausea, to chronic impacts, such as various cancers, birth defects, infertility, and endocrine disruption (Cecchi etal.,2012 and Alavanja et al.,2013). Many believe that the environmental protection agency (EPA)'s methods for testing pesticides are insufficient because they only examine the effects of exposure to pesticides at high doses without conducting research concerning long-term exposure to low doses of pesticides. These studies neglect to base safety levels on real-life situations. Moreover, the tests examine the effects of a single chemical, whereas people are typically contaminated with small amounts of hundreds of pesticides at any one time(Walker et al.,2005). There is a growing list of pesticides that are suspected of being endocrine disrupters, many of which can be found on our food. Pesticides that have been found in recent surveys such as dicofol, endosulfan, dithiocarbamates, malathion and parathion are suspected endocrine disrupters properties. These pesticides have been found on oranges, grapefruit, soft citrus fruit, lemons, apples, tomatoes, strawberries, mangetout, grapes, melons sweet peppers, aubergine, lettuce, pears, carrots, peaches, nectarines, spinach, spring onions (EPA,2008) as well as residue of six pyrethroid insecticides were detected in Chinese cabbage**(** Fangguim et al.,2006). Mixtures of the pesticides significantly enhanced the production of the reactive oxygen species compared to individual pesticide exposures. Pesticide treatment decreased superoxide dismutase, glutathione peroxidase, and catalase activities in human nuroblastoma cell (SH-SY5Y) cells culture enhance lipid peroxidation as well (Jia and Misra 2007**).** Farmers tend to apply pesticides too close to harvest because of lack of adequate knowledge regarding the safe and judicious use of pesticides, potentially contaminating the crop prior to sending their produce to the market **(**Jallow et al.,2017).

This study aimed to survey the pesticides residues contamination in grapes from new civilized farms in Egypt. In addition, investigate the impact of chronic treatment with mixture of the highest frequented detected pesticides on some biochemical and histological changes in liver and pancreas of albino rats.

Materials and Methods

Reagents and chemicals

Reference analytical standards and formulation of the selected pesticides malathion (Malatox 60% WP), diniconazole (Sumi-eight 5% EC) and lambda-cyhalothrin (karate 2.5% EC) were supplied by central agricultural pesticides laboratory (CAPL, Egypt). Acetonitrile, methanol and acetone were hplc-grade, magnesium sulfate, sodium chloride, sodium hydrogencitrate sesquihydrate, sodium citrate, ammonium acetate were pesticide residue analysis grade and formic acid were purchased from sigma Co. (Sigma-Aldrich, USA). Ultra-pure water was obtained by a Milli-Q (Millipore, USA).

Samples

A total of 1kg (*Vilis vinifera*) exported samples were collected randomly from new civilized farms in Egypt in 2014. After the samples were collected, they were transported to the laboratory which, were completely homogenized and processed for analysis. Homogenized grapes sample 15g was placed into 50mL falcon tube, 15mL of 1% acetic acid in acetonitrile was added, then vortex for 1 min at high speed according (Anastassiades et al.,2003). Afterwards, 4g of magnesium sulfate anhydrous and 1.0g of sodium chloride were added, then extract by shaking vigorously on vortex for 5min and centrifuged at 5,000 rpm for 10min. An aliquot of 1mL supernatant was used for clean-up. Samples clean-up have been done using of 2-mL microcentrifuge tubes for QuEChERS, which contained 150mg anhydrous MgSO4, 25mg PSA, and 25mg C18. An aliquot of 2mL was concentrated to dryness.

Chromatographic conditions

GC determination

The gas chromatography (GC) analysis was performed with an Agilent 7890 GC system, coupled with micro electron capture detector GC-µECD was used for determination of pesticides residues, using capillary column HP-5 (30 m × 0.25mm × 0.25µm). Helium was used as the carrier gas at a flow rate 2ml min^{-1}. The following temperature program was employed: initial temperature of 150ºC held for 2 min; increased at 25ºC min-1 to 220, held for 2 minutes; yet another

increase at 5ºC min^{-1} to reach 245ºC. The injector and detector temperature were 225ºC and 300ºC, respectively. The injection volume was 1µl for all standard and samples using splitless injection mode. The residues in the real samples were tentatively identified by comparing the retention times (RTs) of the sample peaks with the RTs of the injected standards. The chromatographic apparatus was controlled by Chemistation software.

Animals and Experiment

Male albino rats *Rattus Norvogious* (4-6) month age weighing between 180-200 gm were supplied by breeding unit of Egyptian organization for the Biology and Vaccine production A.R.E. The animals were housed in a plastic cages, fed *adlibitum*. The rats were housed at 23 ± 2ºC dark/light cycle. All animals were treated according to the standard procedures laid down by OECD guidelines No. 408 (OECD,1998) sub-chronic oral toxicity-rodent 90-days study.

Animals randomly divided into three experimental groups 10 animals each as follows:

Group1 (C): control group supplemented with free access of drinking water.
Group2 (HM): rats supplemented, with drinking water contains a mixture of the tested pesticides in concentration of highest detected levels in grapes for 90 days.
Group3 (LPM): rats supplemented, with drinking water contains a mixture of the tested pesticides in concentration of lower detected levels in grapes for 90 days.

Sampling

Blood has been collected from the retro-orbital plexus vein according to **(Scharmer, 1967)** on heparinized tubes, at the end of the 3rd month of treatment periods, plasma were separated by centrifugation of the blood samples at 3600 rpm for 15 minutes, plasma were kept at -20 ºC for subsequent use. Animals were dissected for liver and pancreas for histopathological and histochemical studies.

Histopathology and Histochemistry

Histopathological and histochemical examination was carried out according(Drury and Wallington 1980**)**. Liver and pancreas tissues were dissected and the tissue samples were fixed in Bouin's solution for 14–18 h, processed in a series of graded ethanol and embedded in paraffin. Paraffin sections were cut at 5 µm thickness and stained with hematoxylin and eosin for light microscopy examination. Other sections were stained with Periodic Acid Schiff (PAS) stain according (Bancroft and Stevens, 1996) The sections were viewed and photographed on an Olympus light microscope (Olympus BX51, Tokyo, Japan) with attachment photograph machine (Olympus C-5050, Olympus Optical Co. Ltd., Japan).

Biochemical assay

Acetylcholinesterase activity was carried out according (Ellman, 1961). Total thiol proteins of plasma were investigated spectrophotometrically at 412 nm using DTNB as the reagent (Hu and Dillard,1994) . Malondialdehyde (MDA) biomarker of Lipid peroxidation of plasma was determined by reaction of thiobarpeturic acid with the end product of lipid peroxidation according to **(**Ohkawa et al., 1979**)** pink color produced by these reactions was measured spectrophotometrically at 532 nm**.** Paraoxonase was determined in plasma by the method established by (Eckerson et al., 1988) .Total antioxidant capacity were determined by **(Koracevic et al.,2001)** .Plasma glucose level were determined according to (Trinder,1959) using the commercial diagnostic kit of stanbio Co., Spain. Plasma amylase level was determined by the method established by (Henry,1974) using the commercial diagnostic kit of boehringer mannheim (Germany). Plasma transaminases (AST and ALT) activities were determined according to (Reitman and Frankel1957) using the commercial diagnostic kit of stanbio Co., Spain. Total plasma insulin level were determined according (Bates,1983**)**using radio immunoassay kit (DPC. Co. American).

Statistical analysis

Data obtained from the biochemical analysis of different groups are represented in tables as Mean ± Standard error (mean ± SE). The significance difference between groups was calculated by one-way analysis of variance (ANOVA) at $p<0.05$ using the SPSS-PC computer software package version 10.

Results

Monitoring Results:

Table 1, showed the highest frequented pesticides which detected in grape samples in 2014. Total 60 samples were analyzed and the obtained results showed that forty five samples (75%) of grape samples were contaminated with these three highest frequented pesticides malathion, lambada-cyhalothrin and diniconazole. Results showed that the pesticides contamination levels of these three pesticides were ranged from (8.5-1.3), (0.3-0.1) and (0.2-0.13) mg/kg for malathion, lambada-cyhalothrin and diniconazole, respectively. The maximum residue limit (MRL) of malathion, lambada-cyhalothrin in grapes has been established by Codex Committee (FAO/WHO joint meeting) to be 8.0 and 0.3 mg kg-1. While FAO/WHO has not established MRLs for diniconazole, However European food safety authority (EFSA) MRL for diniconazole in grapes was 0.2 mg kg-1. The obtained results reported that ten (22 %) samples out of 45 contaminated samples were exceeded the recommended MRL, While, the detected malathion and lambada-cyhalothrin residues levels were below the established maximum residue level (MRL).

Table 1. Highest frequented pesticide residues detected in grapes samples in 2014

Pesticides	MRL (mg/kg)	Maximum detected level	Minimum	No. of contaminated samples	No. of violated samples	% violated samples
Malathion	8.0*	8.5	1.3	45	10	22.2
Lambada-Cyhalothrin	0.3*	0.3	0.1	45	-	-
Dinaconazole	0.2#	0.2	0.13	45	-	-

*MRL= Maximum Residue Limit according to Codex Alimentarius Commission.
#MRL= Maximum Residue Limit according to European Food Safety Authority.

Biochemical results

The depicted data in Table (2) demonstrated that rats treated with high pesticides mixture (HPM) and low pesticides mixture (LPM) have remarkable elevation in plasma malondialdehyde (MDA) ;oxidative stress biomarker; this elevation was pronounced in HPM group (116%) from control compared with (34%) from control in LPM group. This effect concurrent with significant reduction in defense parameters SH-protein and total antioxidant capacity the reduction was remarkable in HPM group than LPM one. Paraoxonase enzyme as one of antioxidant enzyme recorded significant reduction in both treated groups pronounced in HPM than LPM (Table 2). Remarkable significant inhibition in plasma acetylcholinesterase (AchE) in rats of HPM treated group was recorded (-31.8%) from control. However, low LPM induced moderate significant inhibition in AchE (-20.05%) from control at $P<0.05$. Results of liver biomarkers alaninaminotransferase (ALT) and aspartateamintransferase (AST) declared that HPM Induced pronounced induction in both ALT and AST activities significant versus control in HPM and versus control and HPM in LPM at $P<0.05$. Significant elevation in plasma glucose level in both treated groups is a feedback response to the significant reduction in the level of plasma insulin hormone as well as significant reduction in amylase activity at $p<0.05$ (Table 3).

Table 2. Effect of high and low residue levels of pesticides mixture on some oxidative stress markers in plasma of male albino rats.

Groups	AChE (U /ml)	ALT (U/ml)	AST (U/ml)	Glucose (mg/dl)	Amylase (U/ml)	Insulin (µIu/ml)
Control	572.94±37.02	29.65 ±3.11	60.53±2.54	113.15±9.33	2150.5±1128.6	17.32±0.62
HPM	390.57±28.18[a] -31.83%	102.35±5.88[a] 245.194%	215.54±5.68[a] 256.09%	182.95±14.34[a] 61.68%	1676±140.5[a] -22.07%	6.38±1.27[a] -63.16%
LPM	458.03±44.15[a] -20.056%	96.36±2.63[ab] 224.99%	185 .69±8.77[ab] 206.77%	162.76±12.64[a] 43.84%	1896.3±321.3[ab] -11.82%	7.23±0.24[ab] -58.26%

Results were expressed as mean + SE and % change from control of 10 rats.
HPM = high dose (mixture: lambada cyhalothrin, malathion and diniconazole).
LPM = low dose (mixture: lambada cyhalothrin, malathion and diniconazole)
[a]: significance difference versus control at $p< 0.05$
[b:] significance difference versus HM at $p< 0.05$.

Table 3. Effect of high and low residue levels of pesticides mixture on some liver and pancreatic markers in plasma of albino Rats.

Groups	MDA (µmol/dl)	SH-proteins (µmol/dl)	Total Antioxidant Capacity (TAC) (mg/dl)	Paraoxonase (µu/ml)
Control	15.49 ± 1.56	76.99 ± 5.61	2.30 ± 0.1	78.55 ± 11.23
H PM	33.92 ± 0.87[a] 116.7%	49.63 ± 5.41[a] -39.4 %	1.50 ± 0.40 -34.78%	50.07 ± 14.46[a] -36.3 %
LPM	20.85 ± 4.10[ab] 34.5 %	56.03 ± 3.86[ab] - 27.2%	1.8 ± 0.39 -21.74%	60.60 ± 6.57[ab] - 22.9%

Results were expressed as mean ± SE and % change from control of 10 rats.
HPM = high dose (mixture: lambada cyhalothrin, malathion and diniconazole).
LPM = low dose (mixture: lambada cyhalothrin, malathion and diniconazole)
[a]: significance difference versus control at $p< 0.05$
[b:] significance difference versus HM at $p< 0.05$.

Histopathological and Histochemical Results

Liver examination

The control group demonstrated in plate1 showed normal liver section showing the central vein and the cords of hepatocytes are radiating from it. The hepatocytes are polygonal in shape and contain vesicular nuclei and acidophilic cytoplasm, the liver cells cords entrap liver blood sinusoids between them, these sinusoids are lined by sinusoid lining cells (photomicrograph. 1a). The control liver stained PAS with showed normal liver architecture and cells with red coloration of the wall of the central vein representing the glycogen in the blood vessel wall and few spots in the liver cells (photomicrograph. 1b). However, group of high dose mixture (HPM) showed marked dilatation and congestion of the central vein, distortion and interruption of the liver cords, widening of the liver sinusoids and signs of degeneration of the hepatocytes in the form of vacuolated cytoplasm faint and pyknotic nuclei and shrunken cells and intense mononuclear cellular infiltration Plate 2 (photomicrograph.2a). In PAS stained sections depletion of glycogen was noticed from the liver cells and mononuclear cellular infiltration was noticed (photomicrograph. 2b). Also, group of rats treated with low pesticides mixture (LPM) showed dilatation of the central vein less distortion of the liver cords, edema of the liver cells and narrowing of

the liver sinusoids, vacuolation of the cytoplasm of some cells was also noticed. Intense mononuclear cellular infiltration was also noticed (photomicrograph. 2c). In PAS stained section the cytoplasm of the liver cells showed minimal glycogen in some areas, while some cells showed vacuolated cytoplasm. Some nuclei showed faint staining (photomicrograph. 2d).

Pancreas examination

Control group expressed in plate 1 showed the acini of the pancreas with their pyramidal cells and each cell has a round nucleus with the characteristic basal basophilia and apical acidophilia. The acini are divided by thick connective tissue septa into groups. Islets of Langerhans were seen as group of round cells scattered between the pancreatic acini and are rich in blood supply (photomicrograph.1c). PAS stained sections showed only red coloration around the blood vessels (photomicrograph. 1d). On the other hand, increased cellularity of Islets of langerhans increased vascularity, the Beta cells appeared edematous with pale cytoplasm and round prominent nuclei. The acinar arrangement was slightly disturbed (plate3, photomicrograph.3a). PAS stained sections showed no signs of glycogen but increased cellularity of the islets of Langerhans was also noticed (plate3, photomicrograph.3 b). Meanwhile, Group LPM showed normal acini and cells in both H&E and PAS stained sections only increased cellularity of the endocrine cells were seen (plate3, photomicrograph. 3c, 3d).

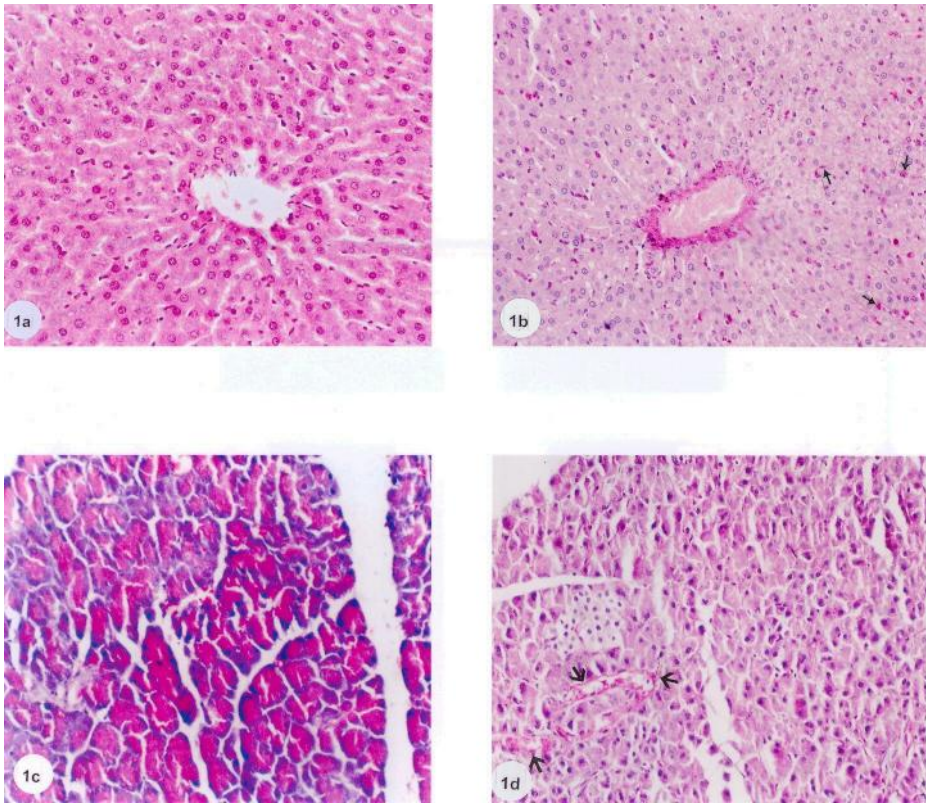

Plate1. (a) a photomicrograph of control liver section showing the central vein and the cords of hepatocytes are radiating from it, b. control liver stained PAS with showed normal liver architecture and cells with red coloration of the wall of the central vein representing the glycogen in the blood vessel wall and few spots in the liver cells (arrows), c. normal pancreas showing closely packed acini with basal basophilia and apical acidophilia, d. PAS stained sections of control pancreas showing only red coloration around the blood vessels (arrows). X 250.

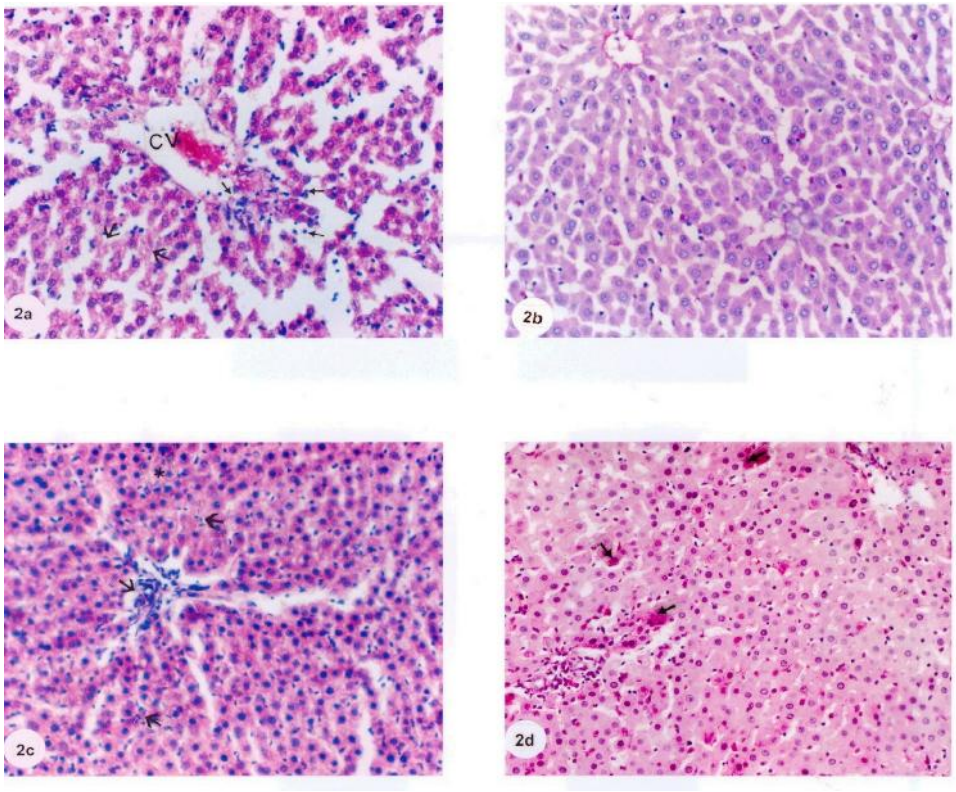

Plate 2. (a) a photomicrograph of liver of group HM showing marked dilatation and congestion of the central vein (CV), vacuolated cytoplasm and degenerated shrunken cells (big arrows) mononuclear cellular infiltration (arrows), b. PAS stained sections of group HM depletion of glycogen was noticed in the liver cells, c. liver section of group MM showing edema of the liver cells, vacuolation of the cytoplasm of some cells (*) and narrowing of the liver sinusoids and intense mononuclear cellular infiltration (arrows), d. PAS stained liver section of group MM showing minimal glycogen in few areas (arrows), e. group LM showing vacuolation of cytoplasm of the liver cells (small arrows), some cells appeared shrunken with absent nuclei (big arrows), f. PAS stained section of group LM slight deposition of glycogen (G). X 250.

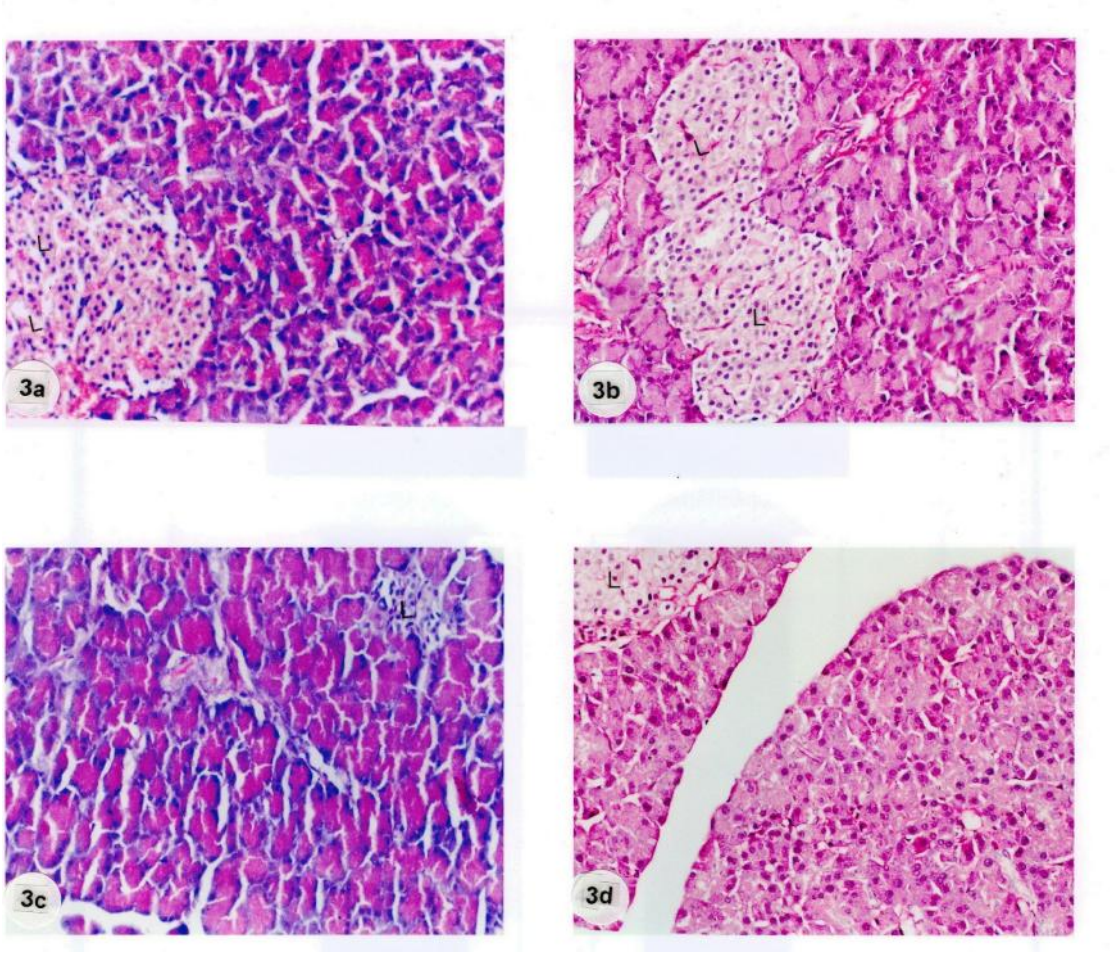

Plate 3. (a) a photomicrograph of section of pancreas of group HM showing increased cellularity of Islets of langerhans, edema of the cells (L), b. PAS stained pancreas section of group HM showing increased cellularity of the endocrine part of the Pancreas, c, d pancreas section of group MM showing normal acini and cells in both H&E and PAS stained sections only increased cellularity of the endocrine cells were seen, e, f Pancreas section of group LM showing No changes from the control in both H&E and PAS staining. X250.

Discussion

Pesticide residues in foods are detected readily in conventionally produced products and fresh fruit and vegetables (Andersen and Poulsen,2001) processed baby foods as well (Stepan et al.,2005). The concentrations detected are usually low, and are often dismissed as being of little concern to human health (Rawn et al., 2004). Different residue levels of organophosphorus insecticide "malathion", pyrethroid insecticide "lambada cyhalothrin" and "diniconazole" fungicide were detected in grapes fruit .The detected pesticides residues refer to direct treatment of the edible commodity and fruit is close to the time of harvest to ensure that wholesome product reaches the consumer(Dogheim et al.,2001). The detected pesticides have different mode of action organophosphates Ops induce inhibition of acetylecholinesterase (AChE) degradation and accumulation of acetylcholine (Abdollahi et al.,1996). However, pyrethroids are potent neurotoxicants that interfere with nerve cell function by interacting with voltage dependent sodium channels as well as other ion channels, resulting in repetitive firing of neurons and eventually causing paralysis (Shafer and Meyer 2004). Wheras, diniconazole induced steroid demethylation (ergosterol biosynthesis) inhibitor (Tomlin.,2004). Pharmacokinetic effects of mixture of these pesticides with different levels in rats revealed significant inhibition in plasma cholinesterase in all treated groups these results run with previous studies significant inhibition to serum cholinesterase in birds treated with combination of organophosphorus compounds (dimethoate, chlorpyrifos, diazinon and malathion) and fungicides (penconazole, propiconazole and prochloraz), the pronounced

inhibition was attributed to an increased activation of the Ops compound to its active oxon form as consequence of induction of microsomal monooxygenase by fungicides (Johonston et al.,1994). Significant inhibition in plasma cholinesterase enzyme in rats treated with different concentrations of mixture of chlorpyrifos and cypermethrin insecticides (Latuszynska et al.,2001). These findings explain the synergistic effect between mixture of (malathion, lambada-cyhalothrin and diniconazole) in high and low dose levels, it must be noted here that the effect was dose dependent. Elevation in oxidative stress biomarker (malondialdehyde) in plasma of treated animals is concomitant with reduction in total thiol protein ant total antioxidant capacity biomarkers in both treated groups. The main mechanism of OPs is irreversibly binding to the enzyme acetylcholinesterase and binding to the activity that results in accumulation and prolonged effect of acetylcholine and consequently with acute muscarinic and nicotinic effects. In chronic and sub chronic exposures added to cholinesterase inhibition, induction of oxidative stress has been reported as main mechanism of toxicity (Abdollahi et al.,2004; Banerjee et al., 2007) . Meanwhile (Ranjbar et al.,2005) reported that 22 acute OPs poisoning patients levels have significant elevation in lipid peroxidation decreased levels of total antioxidant capacity, total thiols, and cholinesterase activity. A significant correlation existed between cholinesterase depressions. Moreover, deltamethrin induced significant inhibition in total thiol-protein (non-enzymatic antioxidant) in fish (Parvez and Raisuddin 2006). Also, induction of oxidative stress in liver and brain were recorded in rats treated with different doses of cypermethrin (Elhalwagy et al.,2015). Mixture of endousulfan and zineb pesticides in human neuroblastoma cells (SH-SY5Y) significantly enhanced the production of superoxide anion and hydrogen peroxide in a dose-and time-dependent manner (Jia and Misra 2007). Strong oxidative and nitrosylative stress quickly and efficiently diminishes GSH level in the cell (Klatt and Lamas 2000). Amylase acts on complex carbohydrate-like starches to yield individual glucose units for energy metabolism. Observed reduction in plasma amylase activities with accumulation in in glycogen in liver and pancreatic tissues was recorded by (Elhalwagy et al.,2015) Hyperglycemia accompanied with hypoisulinemia were recorded in the present study in treated groups, previous studies reported increase in glucose level in rabbits treated sublethal of cyhalothrin(Elhalwagy et al.,2015). mainwhile, Abdollahi et al.,2004). reported that hyperglycemia is one of the side effects in poisoning by Ops. Synergistic effect between mixture of pesticides used in the present study can be hypothysized. Degenerative changes were seen in the hepatocytes in the form of vacuolation of their cytoplasm, absence or faint pyknotic nuclei, also depletion of the glycogen from the liver cells was noted with the mixture of pesticides. These cellular degenerative changes were supported by (Celik et al .,2005). Acute pancreatitis is a well-known complication of organphosphorous poisoning; whose etiology maybe excess cholinergic stimulation within the pancreas and ductular hypertension Harputluoglu et al.,2003, this pancreatitis may cause injury to the pancreatic cells and affects insulin secretion. According to the biochemical results of the current study insulin level has decreased in the blood despite the increased cellularity of the pancreatic islets suggesting dysfunction of those cells in secreting insulin as a reaction to the increase of the plasma glucose level in the rats ingested with the pesticide mixture. (Pournourmohammadi et al.,2007) reported that malathion treated rats secrete lower insulin in the presence of basal or stimulatory glucose concentrations and concluded that malathion induced hyperglycemia is most probably due to disruption of the islets' mitochondrial function due to oxidative stress and the induction of insulin resistance so that it cannot control the hyperglycemia. These findings agree with our histopathological remarks of disturbance in the Beta cells of the Pancreas islets. While Kakkar et al (1998) recorded that the histological alteration could correlate to the parameters related to oxidative stress. Depletion of glycogen in the liver cells was noticed in both rats treated with the mixture especially in the high doses, and this finding was supported by (Yehia et al., 2007) who reported that glycogen content was decreased in liver and increased in kidney Bowman’s capsule with diazinon toxicity in rats. Several studies indicated that hyperglycaemia is temporary, which is probably due to a stimulated glycogenesis that increases hepatic glycogen deposition and return of glucose to control levels and added that one possible explanation could be the turnover of glucose by a succession between its release via glycogenolysis and gluconeogenesis, which involves abnormal hyperglycaemia, and its storage via glycogenesis in subchronic exposure to malation (Rahimi and Abdollahi 2007). suggested that since hyperglycemia induces oxidative stress, it could be concluded that it is a mechanism of oxidative stress in organophosphorous poisoning, moreover(Gearhart and Parbhoo 2006) assumed that stimulation of

sympathetic nervous system during stress leads to release of catecholamines, glucagons and growth hormone which result in promotion of gluconeogenesis, glycogenolysis, insulin resistance and constitution of hyperglycemia.

Conclusion

Pesticides mixture induced significant inhibition of AChE, hyperglycemia that it is a mechanism of oxidative stress induced degeneration in liver and pancreas tissues. Regulatory studies which designed to test one chemical at a time, even though in the real world, animals and people exposed to hundreds of chemicals simultaneously. This gap between real exposure and artificial conditions in the laboratories studies is likely to have led to significant underestimates in risks of chemical exposures. Further studies recommended to study the molecular changes of pesticide mixture in these organs supported with further biochemical measurements.

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