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Alteration of biochemical markers of liver and kidney in rats, following exposure to pesticide mixtures

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ABSTRACT/RESUME

Abstract: *The objective of this study was to highlight the effects of various pesticide mixtures on biochemical markers of liver and kidney (total protein, creatinine, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, serum enzymatic activities of alanine aminotransferase and aspartate aminotransferase) in rats. Three pesticide mixtures commonly used on high consumed fruits and vegetables of Algerian cuisine, were selected. Chlorpyrifos, thiacloprid, spiromesifen, cypermethrin, bromuconazole, abamectin, chlorothalonil, methomyl, spirotetramat, deltamethrin, spirodiclofen, and indoxacarb were administered to male Wistar rats once per day at various doses corresponding to their acceptable daily intake (ADI) value for 120 days.*

The study showed that the exposure to pesticide mixtures induce an oxidative stress assessed by an increase of the concentration of thiobarbituric acid reactive substances (TBARS) in liver and kidney tissues reflecting lipoperoxidation content as well as depletion of enzyme activities as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Moreover, the three pesticides mixture induced liver and kidney dysfunctions. These significant changes were more apparent in mixtures corresponding to most diversified diets.

It can be concluded that pesticide mixtures affect adversely and alter biochemical markers of liver and kidney even at minimal doses.

I. Introduction

The use of pesticides to improve crops has become a common practice around the world. Pesticides used in agriculture for achieving better quality products and controlling vectors of diseases can be extremely toxic and harmful to humans.

Human populations may be constantly exposed to complex pesticide mixtures through their diet. Several studies have indicated that residual amounts of pesticides have been detected in vegetables, fruits and many food products (Curl et al., 2003; john et al., 2001)

Pesticides have been linked to the dysfunctional systems of several organs, including liver and kidney (Bhardwaj et al., 2010; Maalej et al., 2017) which play a major role in pesticide biotransformation. Besides, many studies have been carried out on pesticide effects on biochemical changes (Hernández et al. 2005; Yehia et al. 2007; Hariri et al. 2010).

Otherwise, oxidative stress is defined as a state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of balance between them. It is associated with damage to a wide range of molecular types including lipids, proteins, and nucleic acids (Mc Cord, 2000). Evidence suggests that the formation of oxygen free radicals can be a

major factor in the toxicity of many pesticides (Tariba Lavaković et al., 2017; Igo et al., 2020). When produced in excess, free radicals and oxidants generate a phenomenon called oxidative stress; this process plays a major part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, ageing, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases (Matés et al., 1999; Farag et al., 2000; James and Hall, 2015; Moisan et al., 2015; Rivero et al., 2015). The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced, or externally supplied through foods and/or supplements (Butterfield et Lauderback, 2002; Zarkovic 2003). Pesticides are known to produce oxidative stress by increasing generation of reactive oxygen species and decreasing levels of cellular antioxidants (El-Demerdash, 2011). An increased oxidative stress in certain tissue may lead to a rise in the rate of lipid peroxidation (LPO) which occurs by a radical chain reaction, it spreads rapidly and affects a great number of lipid molecules. Lipid peroxidation is known to be one of the molecular mechanisms for cell injury in pesticide poisoning (Halliwell and Chirico, 1993; Kehrer, 1993; Seth et al., 2001).

Experimental studies have helped to confirm the harmful effects of some pesticides. However, most *in vitro* and *in vivo* studies have investigated the effect of only one pesticide and there is no accepted procedure to assess the risk of cumulative exposure to multiple pesticide residues that cause changes in the toxicokinetics of individual compounds, thus modifying the predicted toxicity (Reffstrup et al., 2010; Jensen et al., 2013). The pesticide formulation is a mixture of active and other ingredients, such as solvent, wetting agents, dispersants and additives. Many papers describe the toxic effects of active ingredients but relatively little information is available on the harmful effects of commercial formulations of pesticides. Therefore, it is necessary to evaluate the toxic effects of the formulated pesticides. The risk assessment should be carried out for mixtures containing compounds found together in food. In consumer exposure to pesticides, formulated pesticides, not only active ingredients, affect human health. A number of evaluations have shown that pesticide formulations contain by-product of their industrial synthesis (Linnainmaa, 1983) and adjuvants (solvents, dilutants, dispersants, emulgators, potentiators) that are kept secret by manufacturers, so all of these chemicals should be considered in researches simulating the exposure. This is why we focused our study on pesticide formulation instead on purified active ingredient.
The acceptable daily intake (ADI) is considered to be a level of intake of a chemical that can be ingested daily over an entire lifetime without any appreciable risk to health. Doses used in the present study correspond to the Admissible daily intake (ADI) for each pesticide (JEFCA, 1962).

Based on these considerations, the aim of the current work was to assess the risk of exposure to pesticide mixtures on biochemical markers of liver and kidney in rats, through food consumption.

II. Materials and methods

II.1. Experimental animals

24 adult male albino rats of Wistar strain (Rattus norvegicus), weighing 180–200 g were provided from Pasteur Institute (Algiers, Algeria). Rats were housed in cages at room temperature of 22 ± 2 °C, kept under standard conditions of a 12 h light/dark cycle and minimum relative humidity of 40% and fed a standard laboratory diet. Animals were acclimatized for 1 week prior to the experiments and were divided into 4 groups of 6 each. During the experiments, all ethical guidelines for care and use of laboratory were followed carefully, maximum care was taken to minimize animal suffering and the number of rats was kept to a minimum.

II.2. Treatment

Rats were randomly divided into four groups, each comprising six animals. Group I served as a control. The remaining three groups II, III and IV represented three consumers categories defined according to their daily consumption of fruits and vegetables. All treated groups received the ADI of each pesticide of the corresponding mixture, once per day, for 120 days in corn oil. The pesticides concentration was calculated depending on the percentage of active ingredients of pesticide commercial formulations and rat weight. Rats were weighed weekly and observed for signs of toxicity during the experimental period. The test substances were administered orally by intragastric tube throughout the experimental period.
Group I: received corn oil by gavage on the same dosing schedule and served as vehicle controls.
Group II: represented consumers of main dish only; received Mixture 1: chlorpyrifos (0.001 mg/Kg of body weight "b.w."/day), thiacloprid (0.01 mg/Kg b.w./day) , spiromesifen (0.03 mg/Kg b.w./day), cypermethrine (0.04 mg/Kg b.w./day), bromuconazole (0.01 mg/Kg b.w./day), abamectin (0.0025 mg/Kg b.w./day), chlorothalonil (0.0015 mg/Kg b.w./day), methomyl (0.0025 mg/Kg b.w./day) and spirotetramat (0.05 mg/Kg b.w./day) formulations at concentrations corresponding to their active ingredient ADI, once per day, for 120 consecutive days.

Group III: represented consumers taking main dish and lettuce; received Mixture 2 = Mixture1 + spirodiclofen (0.015 mg/Kg b.w./day) and deltamethrin (0.01 mg/Kg b.w./day) formulations at concentrations corresponding to their active ingredient ADI, once per day, for 120 consecutive days.
Group IV: represented consumers taking main dish, lettuce and fruits; received Mixture 3 = Mixture 2 + indoxacarb (0.006 mg/Kg b.w./day) formulations at concentrations corresponding to their active ingredient ADI, once per day, for 120 consecutive days.

II.3. Preparation of tissue homogenate

The animals were sacrificed 24 h after the last treatment under light ether anaesthesia. Blood was drawn by cardiac puncture for separation of serum. The liver and kidney were rapidly excised, rinsed with isotonic saline, blotted dry on filter paper and weighed. Then, 10% weight/volume homogenates of tissue were prepared in buffer (0.1 M Tris-HCl buffer (pH 7.4) and centrifuged at 3000 × g for 20 min at 4 °C. Subsequently, the resulting supernatants were collected for biomarkers determination of oxidative stress: superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) and total protein. Relative liver and kidney indices were measured according to the formula:

Relative organ weight (g/100 g) = [Total organ weight (g)/Final body weight (g)] × 100. All assays were performed with freshly isolated samples.

II.4. Serum biochemical parameters

Serum samples were investigated for the concentrations of total protein, creatinine, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and serum enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Serum levels of urea and creatinine were also measured. Parameters were determined spectrophotometrically in serum samples using commercially available kits and following the manufacturer's instructions.

II.5. Oxidative stress biomarkers in liver and kidney

Lipid peroxidation, superoxide dismutase, catalase activity, glutathione peroxidase activity and reduced glutathione were estimated using spectrophotometric methods.

Lipid peroxidation (LPO) was determined by measuring thiobarbituric acid reactive substances (TBARS) content in tissue homogenates according to the method of Uchiyama and Mihara (1978). The TBARS content was measured spectrophotometrically at 532 nm.

Determination of superoxide dismutase (SOD) in tissue was carried out according to Misra and Fridovich (1977). Reaction mixtures contained sodium carbonate, nitroblue tetrazolium and freshly prepared hydroxylamine hydrochloride. The reaction mixtures were blended by inversion followed by the addition of the clear supernatant of tissue homogenates (0.1 ml, 1:10 weight/volume). The changes in absorbance of samples were recorded at 560 nm. The activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 U per milligram of protein.

Catalase (CAT) activity in liver and kidney homogenate was assayed by the method of Aebi (1984). Briefly, 50 ml aliquot of tissue supernatant was added to a buffer (pH 7.0). The reaction was started by addition of 1.0 ml freshly prepared 30 mmol/l H_2O_2. The decomposition rate of H_2O_2 was measured spectrophotometrically at 240 nm for 1 min. One unit of CAT activity was defined as the enzyme amount required to decompose 1 nmol H_2O_2 in 1 min.

The glutathione peroxidase (GPx) activity was determined according to the method of Lawrence and Burk (1976). The change rate of absorbance during the conversion of NADPH to NADP+ was recorded spectrophotometrically at 340 nm for 3 min. The GPx activity for tissues was expressed as mmol GSH oxidized/min per mg of protein.

Reduced glutathione level was determined according to the method of Ellman (1959). The absorbance was measured at 417 nm. Reduced glutathione level of homogenate was calculated using a standard curve. The standard curve was drawn using different known levels of reduced glutathione solution.

II.6. Statistical analysis

All data were expressed as a mean ± standard error (SE). The statistical significance was assessed via Tukey test and one-way analysis of variance (ANOVA) by SPSS. If p values were less than 0.05, the results were regarded as statistically significant.

III. Results and discussion

III.1. Body and relative organ weights of rats

Mortality was not observed in any of the experimental groups during the experimental period. The rats representing exposure to pesticide mixtures according to the three different consumer diets for 120 days showed a significant decrease of the final body weights and relative kidney weights when compared with the control. The relative liver weights did not differ significantly (Figure 1A and 1B).

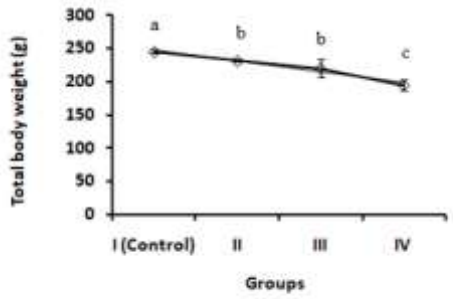

Figure 1A

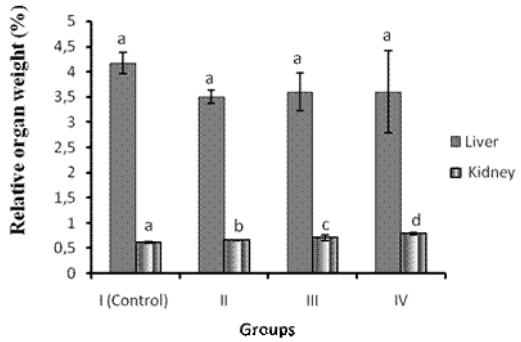

Figure 1B

Figure 1. *Body weight (Fig 1A) and relative weights (Fig 1B) of liver and kidney of male rats exposed to three pesticide mixtures at doses corresponding to their acceptable daily intake value for 120 days.*
Values are expressed as mean ± SE, n = 6. Means with common superscript letters on the same line are not significantly different (p<0.05).

III.2. Liver and kidney function biomarkers

The data on the hepatic and renal function parameters in rats treated with the three mixtures of pesticides for 120 days are presented in Table 1. A significant increase in the activity of AST, ALT and total protein in serum was registered in groups II, III and IV compared to group I. Only AST activity presented a significant difference ($p<0.05$) between groups II and III. Except for total protein, levels of all hepatic markers were significantly different between groups III and IV. There was also a significant difference between groups II and IV for ALT and between all treated groups for AST. All pesticide mixtures caused a significant increase in plasma urea and creatinine levels (Table 1). The comparative analysis of urea and creatinine showed that group II significantly differs from groups III and IV and that urea levels in groups III and IV were significantly different ($p<0.05$).

III.3. Lipid profiles

As shown in Table 2, the total serum cholesterol levels were significantly reduced in rat groups (124.15 ± 2.43, 119.20 ± 1.91, and 113.65 ± 2.05 mg/dl for mixture I, II and III, respectively) when compared to control value (152.41 ± 3.03 mg/dl), which resulted in significant reduction in serum HDL and LDL-cholesterol in all groups treated with pesticides.

III.4. Lipid peroxidation and antioxidant enzyme activities

Analysis of variance demonstrated that groups III and IV presented higher liver and kidney TBARS levels with significant difference when compared to control (Table 3). Besides, there was a significant difference in liver and kidney TBARS levels between all treated groups. As shown in table 3, except for glutathione peroxidase (GPx) in kidney tissue of group III, pesticide mixtures led to a significant decrease in antioxidant enzyme activities (SOD, CAT and GPx) in liver and kidney tissues of groups III and IV compared to control group. No significant difference in GPx and SOD activities in kidney tissue occurred between groups I and II. The difference between groups consisted of a decreased value in groups III and IV (Table 3)

IV. Discussion

In order to simulate human consumer exposure, we fed rats with pesticide mixtures, found in three daily diets, at low doses corresponding to the ADI value for 120 days. Our results have clearly demonstrated the ability of pesticide mixtures to induce oxidative stress in rat liver and kidneys as proved by increased lipid peroxidation (TBARS) and decreased SOD, CAT and GPx activities and accompanying liver and kidney dysfunctions after 120 days of treatment. Our work revealed that rats exposed to pesticides that are found in the most diversified diet are exposed to more substances and are more affected by the treatment.
In our study, the rat body weight and relative kidneys weights of animal treated with pesticide mixtures were markedly lower than that of the control. However, the relative liver weights were not significantly affected by the treatment. These data are in agreement with Abolaji et al. (2016) who reported that co-exposure of rats to

chlorpyrifos and carbendazim caused a significant decrease in the body weight gain and disturbed the relative kidney weights. Following pesticide administration, toxic compounds are transported by the blood to various organs including the liver and kidney where they may eventually cause harmful effects.

The serum AST and ALT activities are important indicators of liver damage. They are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential functions. The observed intensification in groups III and IV may reflect additive effects of these chemicals on markers of hepatic damage. Several studies reported that pesticides may cause changes in activities of ALT and AST enzymes (Gomes et al., 1999; Eraslan et al., 2009). The increase in transaminase activities results from the impairment and necrosis of tissue function and enzymes liberation from the damaged tissues into the circulation (Lasram et al., 2009; Ozer et al, 2008). Concerning renal function, our results showed that the mixtures of pesticides elevated significantly levels of plasma urea and creatinine. Similar changes were reported in rats and rabbits (Yousef et al., 2003; Sankar et al., 2012). Plasma creatinine and urea have typically been used to diagnose kidney injury (Edelstein, 2008). The observed increase in urea and creatinine suggests that the pesticide mixtures are possibly nephrotoxic. The present study has shown that exposure to these toxins may influence total protein levels. This finding was reported by several studies that demonstrated that pesticides cause changes in protein metabolism (Gomes et al., 1999; John et al., 2001). Pesticides usually elevate the cholesterol levels (Lasram et al., 2009). However, in this work, pesticide mixtures decreased the serum cholesterol levels of rats. These reductions are in accordance with results recorded by Hocine et al. (2016) and Al-Attar et al. (2018). These changes may be due to the hepatic bile ducts blockage that stops or reduces cholesterol secretion into the duedonum portion of the small intestine (Zaahkouk et al., 2000). In experimental studies on humans, several authors have reported elevations in triglycerides (Pothu et al., 2019). In this study, pesticide treatment increased the levels of triglyceride. As a result, pesticide exposure may be associated with a proatherogenic lipid profile and therefore it might be considered as a novel risk factor for insulin resistance and cardiovascular disease (Lasram et al., 2009; Acker and Nogueira, 2012).

The results of the current study showed that the concentration of TBARS in liver and kidneys tissues increased with the number of compounds given following the diet diversification. The elevated TBARS concentration in the plasma demonstrates the increased lipid membranes peroxidation. In addition, the three pesticide mixtures cause a decrease in the activities of CAT, SOD and GPx in liver and kidney of treated rats. Furthermore, diminution in the activities of these antioxidant enzymes was accentuated as the number of used pesticides increased, suggesting additive inhibition of defense activity against cellular oxidative damage in rats. Reduced activities of antioxidant enzymes like CAT, SOD and GPx in liver and kidneys reflects the adverse effects of pesticides mixtures on antioxidant system in different tissues. Antioxidant enzymes namely CAT, SOD and GPx are the first defense line against oxidative stress. Changes in these enzyme activities affect the redox status of cells. The decrease in antioxidant defense can be explained by an increased concentration of free radicals, exceeding their capacity to neutralize them. Changes in CAT activity are regarded as a general response to pesticide poisoning (Sayeed et al., 2003). The decrease in the SOD activity in rats could result from the inefficient scavenging of ROS, which might be implicated in the oxidative inactivation of enzymes especially the deleterious effects due to the superoxide radicals accumulation. Several authors confirmed increased lipid peroxidation and decreased cellular antioxidants levels in different tissues of pesticide-exposed animals (Akhgari et al., 2003; Soltaninejad et Abdollahi, 2009; Uzun et al., 2010; Tiwari et al., 2019). Ojha et al (2011) found that chlorpyrifos, methyl parathion and malathion in combination generated oxidative stress in rat tissues as evidenced by accumulation of lipid peroxidation products MDA which was going parallel with a decrease in CAT, SOD and GPx activities in liver, kidney, brain and spleen of rats. The decrease in SOD and GPx activities and increase in LPO could explain the induction of free radicals in chlorpyrifos-treated rats (Abdollahi et al., 2004). The significant increase in blood TBARS level in pesticide manufacturing workers exposed chronically to high pesticide doses as a result of oxidative stress (Ranjbar et al., 2002). Supporting our results there is a report that exposure to chlorpyrifos causes an increase in oxidative stress in the body, as evidenced by enhanced TBARS levels, accompanied by concomitant decrease in the levels of superoxide scavenging enzymes, CAT and GPx in liver, kidney and spleen (Bebe et al., 2003).

V. Conclusion

Our results are consistent with the hypothesis that ADI levels of pesticide mixtures exposure for chronic period result in significant alterations in the

different biochemical parameters. pesticide mixtures induced liver and kidney damage and marked elevations in LPO as well as alterations in antioxidant biomarkers in liver and kidney tissues of Wistar rats. The alterations induced by the mixtures of pesticides were accentuated as the number of pesticides increases.

Exposure to these investigated pesticides could potentially harm human health and the surrounding environment. Further studies are needed to better understand the toxicity mechanisms of these pesticides.

Table 1. *Serum Liver and Kidney function biomarkers (aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, urea and creatinine) in serum of male rats exposed to three pesticide mixtures at doses corresponding to their acceptable daily intake value for 120 days.*
Values are expressed as mean ± SE, n = 6. Means with common superscript letters on the same line are not significantly different (p<0.05).

Groups	AST (U/l) mean ± SE	ALT (U/l) mean ± SE	Total protein (g/l) mean ± SE	Urea (mmol/l) mean ± SE	Creatinine (mmol/l) mean ± SE
I (Control)	118.37 ± 2.20a	25.71 ± 1.82a	15.53 ± 0.35a	10.30 ± 0.48a	0.33 ± 0.02a
II	145.15 ± 5.00b	55.30 ± 6.35b	18.48 ± 0.28b	11.91 ± 0.95b	0.77 ± 0.05b
III	173.75 ± 7.01c	74.04 ± 3.61b	20.27 ± 0.14b	14.24 ± 0.80c	0.99 ± 0.10c
IV	193.43 ± 9.15d	79.89 ± 3.19c	26.82 ± 0.21c	13.30 ± 0.71c	1.20 ± 0.17d

Table 2. *Total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) in serum of male rats exposed to three pesticide mixtures at doses corresponding to their ADI (see table 1) for 120 days.*
Values are expressed as mean ± SE, n = 6. Means with common superscript letters on the same line are not significantly different (p<0.05).

Groups	Total cholesterol (mg/dl) mean ± SE	Triglycerides (mg/dl) mean ± SE	HDL-C (mg/dl) mean ± SE	LDL-C (mg/dl) mean ± SE
I (Control)	152.41 ± 3.03a	131.46 ± 1.11a	86.50 ± 1.47a	29.62 ± 2.02a
II	124.15 ± 2.43b	143.68 ± 2.45a	66.26 ± 2.63b	29.15 ± 4.23a
III	119.20 ± 1.91b	145.80 ± 3.14a	57.32 ± 1.18c	32.72 ± 1.97b
IV	113.65 ± 2.05b	145.32 ± 2.32a	50.26 ± 2.05d	32.72 ± 1.12b

Table 3. *Total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) in serum of male rats exposed to three pesticide mixtures at doses corresponding to their ADI (see table 1) for 120 days.*
Values are expressed as mean ± SE, n = 6. Means with common superscript letters on the same line are not significantly different (p<0.05).

Groups	LPO(nmol/mg protein)		GPx (U/mg protein)		SOD(U/mg protein)		CAT (nmol/min/mg protein)	
	Liver mean ± SEM	kidney mean ± SEM	Liver mean ± SEM	kidne mean ± SEM	Liver mean ± SEM	kidney mean ± SEM	Liver mean ± SEM	kidney mean ± SEM
I(Control)	70.86 ± 1.99^{a}	65.50 ± 3.57^{a}	11.08 ± 0.84^{a}	7.36 ± 0.41^{a}	8.09 ± 1.03^{a}	16.74 ± 1.52^{a}	374.61 ± 5.88^{a}	169.65 ± 7.78^{a}
II	77.30 ± 3.83^{a}	66.69 ± 3.21^{a}	9.11 ± 0.91^{b}	$6.92 \pm 1.67^{a,b}$	5.73 ± 1.04^{b}	14.75 ± 1.84^{a}	351.48 ± 4.25^{b}	154.82 ± 1.81^{b}
III	92.26 ± 5.92^{b}	84.57 ± 2.99^{b}	8.43 ± 0.57^{b}	$5.44 \pm 0.39^{a,c}$	5.49 ± 0.91^{b}	11.68 ± 1.10^{b}	312.99 ± 6.99^{c}	151.54 ± 3.27^{b}
IV	104.54 ± 8.04^{c}	115.40 ± 4.64^{c}	5.97 ± 0.73^{c}	$4.52 \pm 0.56^{c,e}$	3.38 ± 0.80^{c}	10.73 ± 1.18^{b}	285.04 ± 3.79^{d}	140.68 ± 0.29^{c}

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