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Original Article

Exposure to difenoconazole, diclofop-methyl alone and combination alters oxidative stress and biochemical parameters in albino rats

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Abstract: The herbicides diclofop-methyl and the fungicide difenoconazole are widely used in agriculture and may lead to serious toxicity risks. However, limited studies have been done to evaluate differences in the metabolic effects of these herbicides. Difenoconazole (10 mg/kg) and Diclofop-methyl (1 mg/kg) were orally administrated individually (Groups 1 and 2 respectively) as well as combined (G3) to rats for 28 days. In all treated groups, alanine aminotransferase (ALT) and urea were significantly higher than the control group. Plasma creatinine was also significantly higher in groups G1 and G2 than control. Significant inhibition in gamma glutamyltransferase (γGT) was observed in all treated groups, in addition to significant inhibition of plasma acetylcholinesterase enzyme (AChE) in G3 ($p < 0.01$). There was no effect in aspartate aminotransferase (AST) and albumin. Total plasma triiodothyronine (T_3) hormone was significantly higher in groups G2 and G3 ($p < 0.01$), but significantly lower in G1 group as compared to control. Thyroxin (T_4) was significantly lower in all treated groups than control. Cholesterol level was significantly lower in G3 than control, and a total protein (TP) was significantly higher in all treated groups than control. No differences were observed in glucose levels. Malondialdehyde (MDA) and superoxide dismutase (SOD), an oxidative stress biomarker, was significantly increased in all treated groups comparing to control. Sulphur containing protein (SH-protein) was significantly lower in G1 than control. No significant changes were observed for GST in all treatments. The significant differences in measured biomarkers after application of diclofop-methyl, difenoconazole individually and combined indicate that the investigated pesticides may have potentially harmful effects on humans and the surrounding environment. We suggest that larger studies be conducted to better understand the toxicity mechanisms of these pesticides.

Keywords: Oxidative stress, biomarkers, acetylcholinesterase, diclofop-methyl, difenoconazole, stress markers

Introduction

Chemical compounds, which are used for controlling all kinds of pests and weeds, are continuously required for global food production, but they remain as residues in food, air, and water [1-4]. This widespread use of chemicals results in continued exposure of humans to these chemicals and their residues. Low-level exposure is known to produce a variety of biochemical changes, such as alteration of target cell receptor binding proteins, DNA mutation generation, as well as induction/inhibition of enzymatic activities [5].

Diclofop-methyl, (methyl 2-[4-(2, 4-dichlorophenoxy) phenoxy] propanoate) a phenoxyherbicide, is a selective post-emergence graminicide in the phenoxy propionate group of herbicides, developed for control of wild oats, wild millets, and other annual grass weeds [6, 7]. Very few data are available on the environmental impact

of diclofop-methyl. Diclofop-methyl might affect the fatty acid synthesis by acetyl CoA carboxylase (ACCase) inhibition, cell membrane destruction, assimilates translocated to roots prevention, chlorophyll content reduction, as well as photosynthesis and meristem activity inhibition. It is absorbed primarily through the leaves, and partly through the roots in moist soil. Total annual domestic usage of diclofop-methyl is approximately 750,000 pounds of active ingredient [8]. Long term exposure to high concentrations of diclofop-methyl may cause abnormal changes in lung function, i.e. pneumoconiosis. Prolonged contact with chlorinated biphenyl ethers may cause skin irritation, weight loss and liver injury [9, 10].

Difenoconazole, 1-[2-[2-chloro-4-(4-chlorophenoxy) phenyl]-4-methyl-1, 3-dioxolan-2-ylmethyl]-1H-1, 2, 4-triazole, is a systemic fungicide with novel broad-range activity protecting the yield and crop quality by foliar application or seed treatment. This fungicide is a steroid demethylation inhibitor acting mainly on the vegetative stage of fungi by blocking the mycelial growth either inside or on the surface of the host plant [11]. Difenoconazole (triazole derivative) and diclofop (phenoxypropionic acid derivative) are widely used for crop protection in most countries. Hence, consumers can be exposed to their residues in food and water and farm workers can be exposed during application [12]. Incorrect application and failure to use protective gears while applying fungicides are responsible for irritant injuries to skin and mucous membranes, as well as dermal sensitization [13]. Agricultural pesticides are often used in combination to protect crops. Fungicides are often used in combination with other pesticides and carriers or solvents which may be more toxic [14]. The combined effect across different classes of pesticides is more difficult to predict [15]. Few studies have begun to characterize the toxicological effects of pesticides mixture exposure [16-18].

The purpose of this investigation is to determine and compare the effects of difenconazole (triazole derivative fungicide), diclofop-methyl (phenoxypropionic acid derivative herbicide), and the joint effect of both on the biochemical activities in liver, kidney and thyroid gland in adult male albino rats and to determine residual levels of the two compounds in the rat livers and kidneys.

Materials and methods

Materials

The formulations and active ingredients of used pesticides; diclofop-methyl (Iloxan 36% EC was obtained from Samtrade Ltd, Egypt) and difenconazole (Score 25% EC was obtained from Syngenta Ltd, Egypt). All other chemicals used in this study were of analytical grade and purchased from Sigma (Sigma, USA).

Animals

Twenty adult males Wistar albino rats (*Rattus norvegicus*), (10 weeks old, weighing 210±10 grams), were obtained from the Egyptian Organization for Biological Products and Vaccine, Egypt. Animals were housed under controlled environmental conditions (23±2°C and a 12-hour light/dark cycle). The rats were allowed free access to laboratory diet and tap water. Rats were acclimatized for one week prior to the start of experiment. All animals were treated according to the standard procedures laid down by OECD guidelines and EPA protocol [19, 20], for repeated dose oral toxicity study in rodents. The protocol of this study was approved by the Department of Mammalian Toxicology, Pesticide Central Laboratory, Agriculture Research Center, Egypt.

Experimental design

Male albino rats were divided into four groups of 5 each as per the following details. The first group (G1) was treated with the lowest observed effect level (LOEL) of difenoconazole (10 mg Kg^{-1} body weight [bw]). The second group (G2) was treated with LOEL of 1 mg Kg^{-1} bw of diclofop-methyl. The third group (G3) was treated with a mixture of difenoconazole and diclofop-methyl at concentration 10 and 1 mg Kg^{-1} bw, respectively. The fourth group (C) served as control. 1 ml of the reconstituted pesticides was orally administered to all groups. Injections were freshly prepared prior to application, and treatment was continued for four weeks, 5 times/week. The treatment doses were re-adjusted according to changes in the animals' body weights.

Sampling

Blood samples were collected from the retro-orbital plexus vein according to Schalm *et al*,

[20] into heparinized tubes at the end of the 28-day treatment period. Animals were sacrificed by cerebral dislocation. Plasma samples were separated by centrifugation of the blood samples at 3600 rpm for 15 min in a refrigerated centrifuge at 4°C. Plasma samples were kept at -20°C and analyzed within less than 48 hours.

Biochemical analysis

Plasma transaminases (AST and ALT) activities were determined [22] using an assay kit (BioMerieux Sa, USA). γ-glutamyl transferase (γ-GT) activity was determined [23] according of Szasz kinetic method (Stanbio Laboratory, Spain). Plasma acetylcholinesterase (AChE) activity was assayed by the method of Ellman *et al*, [24]. Albumin and urea levels were determined [25] by calorimetric method of Doumas *et al* and Fawcett and Scott, respectively (BioMerieux Sa, USA). Plasma creatinine was determined by the calorimetric method of Schirmeister (Randox Labororatories, UK) [27].

The plasma level of cholesterol and glucose were determined using an analytical kit (BioMerieux Sa, USA). Standard and quantitative assay for determination of total protein content done based on the method of Bradford [30] by using bovine serum albumin (Sigma) and Commasic Brillant Blue. Plasma Tri-iodothyronine (T_3) and Thyroxine (T_4) hormones were performed using radioimmunoassay kit (DPC Co., USA), by the method of Britton *et al* [31]. The oxidative stress biomarkers were determined by the following methods: Plasma malondialdehyde (MDA) lipid peroxidation biomarker was measured according to Okhawa *et al*, [32] after incubation for 30 minutes at 95°C with thiobarbituric acid in aerobic conditions (pH 3.4). Color change was measured on spectrophotometer at 532 nm. Plasma total SH-proteins was determined by spectrophotometer at 412 nm using DTNB as a reagent according to Hu and Dillard [33]. Plasma glutathione-S-transferase (GST) activity was determined using the method of Habig *et al*, [34] (1-Chloro-2,4-dinitrobenzene (CDNB)-GSH conjugate formed at 37°C and assayed at 340 nm on spectrophotometer). Total superoxide dismutase (SOD) activity was measured using the method of Misra and Frindovich [35]. This method is based on superoxide anion generation by pyrogallol, at room temperature. Superoxide anions reduce Nitro Blue Tetrazolium to blue formazans. SOD catalyses dismutation of radicals and inhibits the formation of formazans. One unit of SOD was defined as the amount of the enzyme which inhibits the rate of the photoreduction by 50%.

Residual analysis

One gram of homogenate tissue was placed in a 90 mm glass mortar with 4 g of anhydrous magnesium sulfate and gently grounded to obtain a homogeneous material. The $MgSO_4$ -tissue mixture was transferred to a clean 50 ml polypropylene centrifuge tube. 10 ml acetonitrile with 1% acetic acid was added before centrifugation at 4000 rpm for 10 minutes [36, 37]. Afterwards, 3 ml of the supernatant were transferred to a clean 5 ml centrifuge tube containing 450 mg of $MgSO_4$ and 125 mg of Primary Secondary Amine (PSA), hand-shake for 30 s, sonicated for 1 minute and centrifuged for 5 min at 4000 rpm [38]. The final extract (2 ml) was evaporated to dryness under a gentle stream of N2 for analysis.

The stock solution of diclofop-methyl and difenoconazole was prepared in acetonitrile at 1 mg/ml. Working solutions were prepared by dilution with acetonitrile and calibration curves were generated by plotting peak area versus concentration. Standard calibration curves presented excellent linearity with regression coefficient $r > 0.998$ and 0.982 with good separation and repeatability for diclofop-methyl and difenoconazole, respectively. The calibration curve of six different concentrations (0, 0.1, 0.5, 1, 5 and 10 μg g^{-1}) were analysed individually for each herbicide and recovery study were repeated three times. The mean recovery values obtained from spiked samples with two levels (0.1 and 1.0 μg g^{-1}) of tested pesticides standards ranged from 90-93% for diclofop-methyl and 85-91% for difenoconazole, respectively.

Agilent 7890 (USA) gas chromatography coupled with electron capture detector GC-ECD was used to determine diclofop-methyl residues, using capillary column HP-5 (30 m×0.25 mm×0.25 μm). Nitrogen was used as the carrier gas at a flow rate of 2 ml/min. The following temperature program was employed: initial temperature of 180°C held for 1 min; increased from 25°C min^{-1} to 220, held for 2 minutes and

Table 1. Physiological parameters in plasma in rats treated with difenoconazole, diclofop-methyl and their mixture for 28 days

	Control	G1	G2	G3
ALT (U/L)	42.38±1.39	67.16±3.91**	58.60±3.35**	45.57±2.16
AST (U/L)	124.26±8.54	128.28±12.61	151.08±5.53	151.62±7.37
GGT (U/L)	6.87±0.46	2.99±0.21**	4.15±0.39**	3.56±0.34**
AChE (U/L)	324.74±19.96	307.40±18.17	311.59±21.05	234.97±19.96**
Albumin (g/dl)	1.91±0.19	1.62±0.15	1.90±0.12	1.81±0.16
Urea (g/dl)	39.125±3.57	57.90±1.41**	78.46±4.81**	73.68±4.76**
Creatinine (mg/dl)	0.47±0.02	0.68±0.03**	0.71±0.06**	0.55±0.02

G1: Rats treated with Difenoconazole, G2: Rats treated with diclofop-methyl, G3: Rats treated with mixture, **: $p \leq 0.01$ compared with control group. All data were expressed as mean±SE from 5 animals.

another increase at 3°C min^{-1} to reach 245°C. The injector temperature was 220°C. The injection volume was 1 µl for all standards and samples. The Agilent-1100 (Agilent, USA) high performance liquid chromatography (HPLC) system consisting of a quaternary pump system, diode array detector (DAD) was used for the determination of difenoconazole. Chemistation software was used to control the LC components and to process ultraviolet data. C18 analytical column (150 mm×4.6 mm, 5 µm) was used. Solvent A (water) and solvent B (acetonitrile) were combined on isocratic conditions (30-70 A: B v/v). The flow rate was 0.7 ml/min, and the column heater was set at 25°C. The investigated analysts were eluted within 5 min. Difenoconazole residue was monitored at the absorbance wavelength of 200 nm.

Statistical analysis

Data was analyzed using SPSS version 17.0 for Windows. Prior to parametric tests, Kolmogorov-Smirnov test was used to evaluate data for normality. All values presented in the text were expressed as mean±standard error (SE). Analysis of variance (ANOVA) one-way test [39, 40] was used to compare differences between groups. Least Significant Differences (LSD) test was used to identify all biochemical parameters that differed between groups. Significance was set at $p \leq 0.05$. Joint action analysis for treatments was calculated using relative interaction index (RII) according to Mansour et al, [41], using relative interaction index (RII) according to the following equation:

$$\text{Interaction index} = \frac{\text{mixture value} + \text{control value}}{\text{lvalue individual compounds in the mixture}}$$

Where;

a. In cases of positive effect on the baseline values (increase or more release of an enzyme activity): I.I.=1 for additive effect, ≥ 1 for potentiation, or ≤ 1 for antagonism;

b. In cases of negative effect on the baseline values (decrease or inhibition of an enzyme activity): I.I.=1 for additive effect, ≤ 1 for potentiation, or ≥ 1 for antagonism.

Results

Biochemical parameters

Table 1 shows that low dose treatment with difenoconazole and diclofop-methyl induced higher levels of plasma liver biomarker enzymes (ALT) in all groups, specifically in G1 and G2 than controls ($p < 0.01$). Furthermore, significantly decreased levels in plasma γ-gamma glutamyl transferase (γ-GT) in all treated groups versus controls were recorded (p-values < 0.01) and a significantly lower mean plasma acetylcholine esterase (AChE) in G3 than other groups including controls (p-values < 0.01). Meanwhile, kidney function biomarkers, urea and creatinine showed significantly higher levels in all treated groups versus controls (p-values < 0.01). There was no significant difference in albumin concentrations between groups.

Table 2 shows significantly higher total plasma mean triiodothyronine (T_3) levels in G2 and G3 treated groups than others (p-values < 0.01). On the other hand, mean plasma thyroxin (T_4) was significantly lower in all treated groups (p-values < 0.001). A significantly lower mean plasma cholesterol was observed in G3 as compared to other groups (p-values < 0.01), while no differences among groups were recorded in glucose levels. Finally, the plasma mean total proteins (TP) was significantly high-

Table 2. Stress markers in plasma in rats treated with difenoconazole, diclofop-methyl and their mixture for 28 days

	Control	G1	G2	G3
T3 (ng/dl)	44.40±3.80	24.09±1.73	135.92±11.74**	118.96±4.79**
T4 (ng/dl)	9.84±0.56	3.99±0.31**	5.11±0.47**	5.17±0.39**
Cholesterol (mg/dl)	37.11±2.27	41.57±3.55	38.22±2.91	28.30±2.62*
Glucose (mg/dl)	217.92±14.07	237.64±19.40	235.58±20.14	202.75±12.94
T. Protein (g/dl)	5.01±0.27	8.09±0.67**	6.73±0.52*	6.78±0.60*

G1: Rats treated with Difenoconazole, G2: Rats treated with diclofop-methyl, G3: Rats treated with mixture, *: significant differences at $p \leq 0.05$, **significant differences at $p \leq 0.01$. All data were expressed as mean±SE from 5 animals.

Table 3. Oxidative stress markers in plasma in rats treated with difenoconazole, diclofop-methyl and their mixture for 28 days

	Control	G1	G2	G3
MDA (mmol/dl)	4.05±0.31	11.78±1.06**	8.94±0.73**	15.83±0.31**
SH protein (mg/dl)	49.74±4.60	33.20±1.99**	45.02±3.82	51.41±2.73
GST (mmol/min/ml)	448.37±14.68	396.47±38.82	496.39±16.97	420.96±30.18
SOD (U/ml)	128.6±10.17	373.54±28.62**	269.75±14.67**	285.63±14.98**

G1: Rats treated with Difenoconazole, G2: Rats treated with diclofop-methyl, G3: Rats treated with mixture, **: significant differences at $p \leq 0.01$. All data were expressed as mean±SE from 5 animals.

er in all treated groups than controls (p-values < 0.05).

As far as the oxidative stress biomarker, MDA levels were significantly higher in all treated groups compared than controls (**Table 3**). This difference is in parallel with the significantly lower plasma total thiol containing protein (SH-protein) in G1 group only versus control at $p < 0.01$. Despite the weak alteration in GST activity in plasma of G1, G2 and G3 treated animals, there was a significantly higher SOD activity in all treated groups than controls (p-values < 0.01).

Joint action analysis

The data obtained from **Tables 1-3** were considered as cases of positive effect on the baseline values (increase or more release of an enzyme) were found with ALT, urea creatinine T_3, total protein, MDA, SH-protein and SOD. While the data of the other biochemical measurements (ã-GT, AChE and cholesterol) were considered as cases of negative effects on the baseline values (decrease or inhibition of an enzyme activity).

Table 4 presents the joint action analysis and interaction index of the mixture of difenoconazole and diclofop-methyl on the different studied biochemical parameters. In the case of positive effects: (I.I.=1 for additive effect, ≥ 1 for potentiation, or ≤ 1 for antagonism) but in case of negative effects: (I.I.=1 for additive effect, ≤ 1 for potentiation, or ≥ 1 for antagonism). Generally, the tested mixture showed different types of interaction with measured biochemical biomarkers according to the following:

1. Potentiating interaction was found in acetyl cholinesterase, cholesterol and SH-Protein.

2. Antagonistic interaction was found in some biochemical biomarkers including liver, kidney, thyroid function and some stress and antioxidant biomarkers such as ALT, γ-GT, creatinine, urea, T_4, total protein and SOD.

3. Additive interaction was found in T_3, and MDA.

Residue levels analysis in liver and kidneys

Table 5 shows that difenoconazole residues were below the limit of detection (0.01 μg g^{-1}) in both liver and kidney tissue samples alone (G1) or in mixture with diclofop-methyl (G3). On the other hand, diclofop-methyl was detected in liver and kidney tissues of treated male rats (G2), at levels 1.17 and 0.5 μg g^{-1} for liver and kidney tissues, respectively. In addition, only

Table 4. Joint intoxication on different studied parameters with each of difenoconazole fungicide and diclofop-methyl herbicide and their mixture

Parameters	Effect	Interaction Index (I.I.)	Joint action
Triiodothyronine (T_3)	Increase	1.00	Additive
Thyroxine (T_4)	Decrease	1.65	Antagonism
Cholesterol	Decrease	0.82	Potentiation
Total protein	Increase	0.80	Antagonism
Alanine aminotransferase	Increase	0.70	Antagonism
Gamma glutamyl transferase	Decrease	1.46	Antagonism
Acetylcholinesterase (AChE)	Decrease	0.90	Potentiation
Urea	Increase	0.83	Antagonism
Creatinine	Increase	0.73	Antagonism
Malondialdehyde (MDA)	Increase	0.98	Additive
SH protein	Increase	1.29	Potentiation
Superoxide dismutase (SOD)	Increase	0.64	Antagonism

Interaction Index=(Mixture value+Control value)/$\sum$ values of individual compounds in the mixture.

Table 5. Residue levels of difenoconazole, diclofop-methyl and in liver and kidney of ratstreated for 28 days

	G1	G2	G3	
	Difenoconazole	Diclofop-methyl	Diclofop-methyl	Difenoconazole
Liver	nd	1.17	0.31	nd
Kidney	nd	0.5	0.17	nd
Sum $\sum$	-	1.67	0.48	-

G1: Rats treated with Difenoconazole, G2: Rats treated with diclofop-methyl, G3: Rat treated with mixture, -: not treated and nd: not detected under experimental conditions.

diclofop-methyl was detected at levels of 0.31 and 0.172 μg g^{-1} in the liver and kidney tissues collected from rats treated with the mixed pesticides, respectively.

Discussion

In the present study, rats treated with each compound individually induced elevation in ALT enzyme levels. These results are in agreement with the previous report that recorded an induction in hepatotoxicity and elevation in liver biomarker enzymes in male Fischer rats treated with 0.6 or 1.0 mmol/kg of 3-(3, 5-dichlorophenyl)-2,4-thiazolidinedione (DCPT) [42]. Moreover, Diuron herbicide exposure induced slight increases in serum AST and ALT levels confirming increased hepatic activity. Our results also showed a significant reduction in γ-GT levels in all treated groups.

The present results also revealed higher urea levels in all treated groups, while the plasma creatinine was higher in groups G1 and G2. These results are consistent with the findings of Christine *et al*, [42] in Fischer rats treated with econazole fungicide. Taybe *et al*, [43] observed that administration of 2, 4-dichlorophenoxyacetic herbicide in adult rats provoked a reduction in glomerular filtration rate objectified by the reduced creatinine clearance and a decline of creatinine excretion consistent with renal failure.

Endocrine disruptors, which are mainly lipophilic herbicides, insecticides, fungicides and related compounds have been widely produced and distributed to improve agricultural production. These chemicals can bioaccumulate in the lipids of organisms in the environment and have relatively high coefficients for adsorption into sediments and soils [44, 45].

The higher total plasma T_3 hormone in both of G2 and G3 treated groups and lower T_4 in all treated groups are in agreement with a previous report by Wolf *et al*, [46] that rats treated with triadimefon induced thyroid follicular cell hypertrophy, increased follicular cell proliferation and colloid depletion accompanied by decreased T_3 and T_4 serum levels with no increase in TSH levels [46]. A study reported that chronic treatment with econazole induced degeneration in thyroid gland function and tissue structure [47]. Furthermore, T_4 levels drastically decreased after three daily injections of dichlorophenol-nitrophenyl ether herbicide (NIT) at 1000 mg/kg/day, while T_3 levels and body weight index (BMI) were relatively unaffected even in the highest dosage group [48]. These observations were also confirmed in the present study as reflected by the alteration in several markers influenced by thyroid activity like cholesterol (decreased in G3) and total proteins (increased in all treated groups).

Our findings also showed that exposure to difenoconazole (G1) and diclofop-methyl (G2) induced significant elevations in MDA and reduction in total thiol proteins (SH-protein).

Numerous works have shown that increased circulating free radicals can be due induced by phenoxyacetic acid herbicides derivatives [49-51]. Lipid peroxidation has been suggested as one of the molecular mechanisms involved in 2, 4-dichlorophenoxyacetic herbicide induced toxicity [43]. Also, bromuconazole-induced toxicity could be attributed to the production of reactive oxygen species as well as depressing endogenous antioxidants and enhancing lipid peroxidation [52, 53]. Previous gene expression analysis of liver tissues from propiconazole-treated mice identified genes and pathways involved in oxidative stress, suggesting that oxidative stress may play a role in propiconazole-induced toxicity [54].

Our results revealed that there was potentiation interaction recorded in acetyl cholinesterase, whereas antagonistic interaction was found in ALT, γ-GT, creatinine, albumin, urea, T_4, cholesterol, glucose and total protein. Furthermore, additive interactions were found in AST, T_3, MDA and GST. Humans may get exposed to one or more pesticides through food intake. However, workers in pesticide manufacturing and packing units and agricultural workers who prepare, mix, and apply pesticides in the fields are directly exposed to more than one pesticide on the same or on successive days [55]. Mansour *et al*, [56], recorded the tested mixture might be of lower "toxic insult" and be safer than other illegal mixed applications of pesticide.

Results of many investigated pesticides indicate that toxins are absorbed and carried to the liver, where the metabolizing enzymes responsible for both the bio-activation and detoxification are present [57]. In our study, diclofop-methyl residues was determined using highly specific detector (ECD) which increased the probability of detection even at very low concentrations of this pesticides in different tissues. The herbicide difenoconazole might be present in concentrations below the limit of detection using HPLC-DAD. Syngenta Ltd. study reported that difenconazole is rapidly and totally eliminated in urine and feces after oral administration [58]. Residues in tissues were not significant and there was no evidence for accumulation [59]. The residual levels of diclofop-methyl detected in both liver and kidney in the present study support the findings of Bayer Ltd which reported that when diclofop-methyl was fed to rats, about 90% is recovered unchanged in feces and urine in 2 days, and about 99% after 7 days [60]. Accumulation of residues of diclofop-methyl in the body was unlikely, and low levels of total residues were found in organs and tissues 7 days after a single dose of 1.8 mg/kg b.w [59].

Conclusion

In conclusion, treatment of male albino rats with the fungicide difenoconazole alone and the herbicide diclofop-methyl alone induced significant alterations in the different biochemical parameters. The combined effect of the two pesticides also had varied effects on most investigated parameters. The study clearly showed an inhibition of AChE activity, suggesting this enzyme as a good indicator of intoxication of erythrocytes by both pesticides. The study also demonstrated an increase of lipid peroxidation in rats exposed to diclofop-methyl and difenoconazole and their mixture. 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.

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Abbreviations

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; γGT, Gamma glutamyl transferase; AChE, Acetylcholinesterase; T_3, Triiodothyronine; T_4, Thyroxine; TP, Total Proteins; MDA, Malondaldehyde; GST, Glutathione S transferase; SOD, Super-Oxide dismutase; Ad, Additive; An, Antagonism; Po, Potentiation; SH protein, sulphur containing protein; GC, Gas chromatography; HPLC, High performance liquid chromatography.

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