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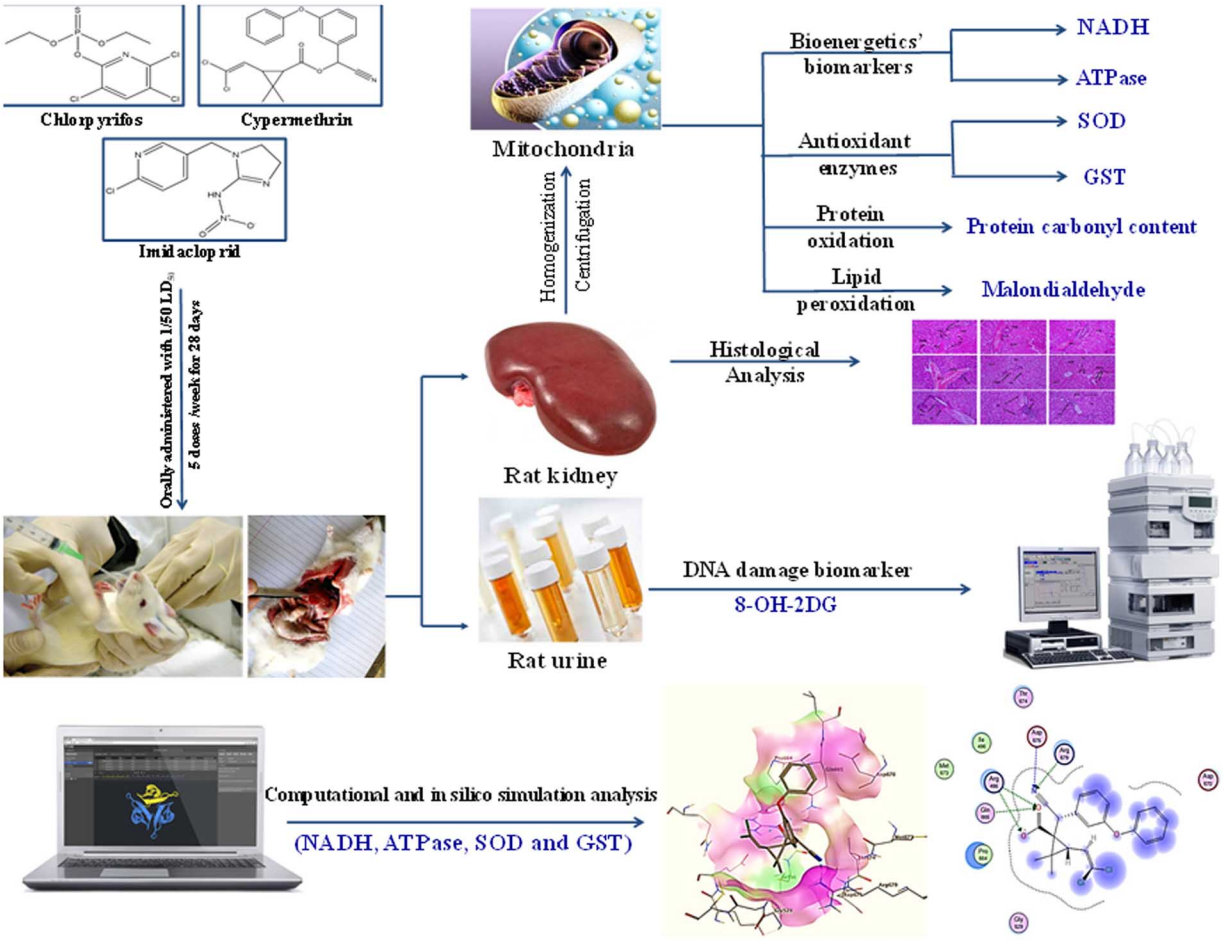

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Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

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B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

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RESEARCH ARTICLE

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Effects of sub-chronic exposure of male albino rats to chlorpyrifos, cypermethrin, and imidacloprid on mitochondrial dysfunction and oxidative stress in the kidney with molecular docking

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List of Abbreviations;

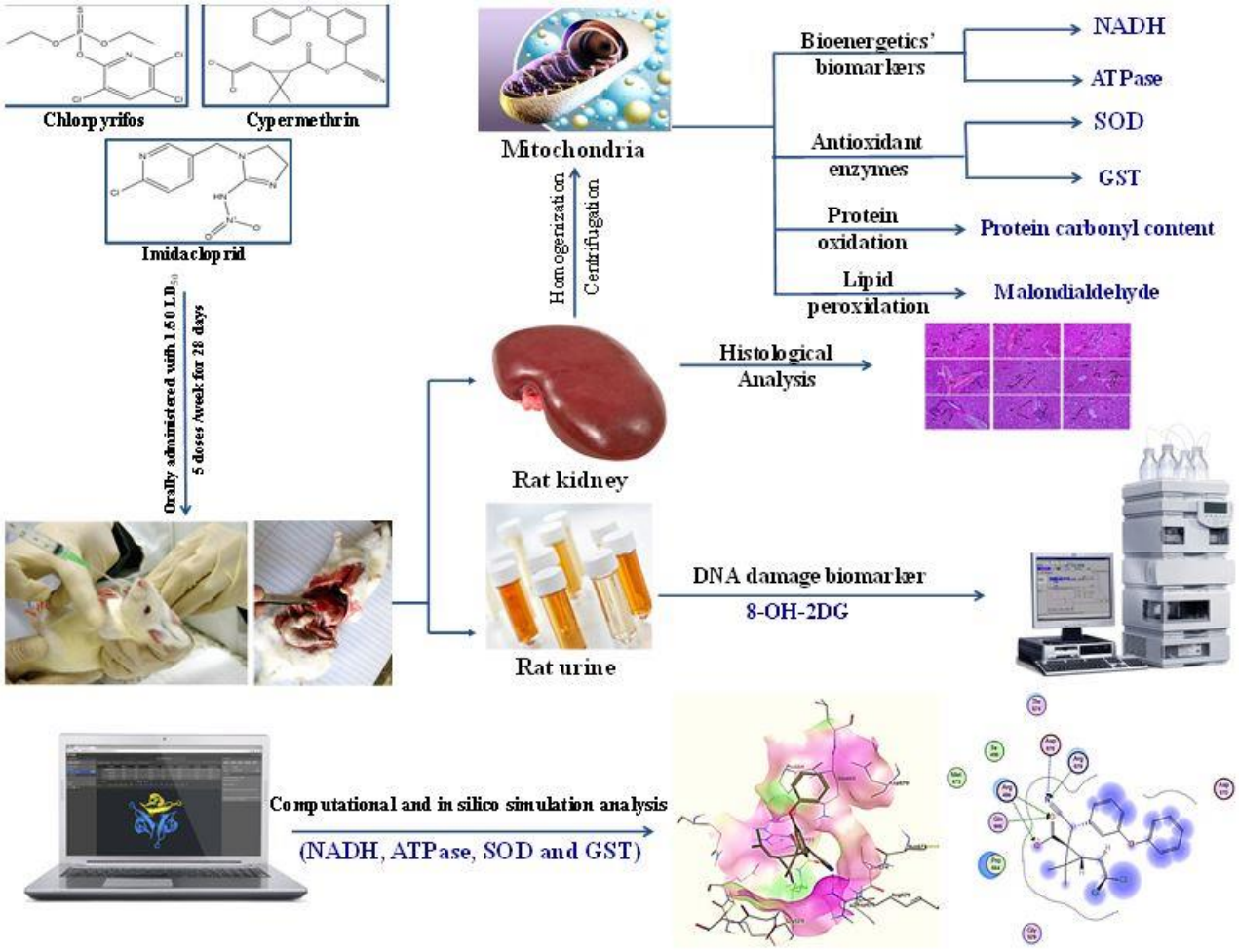
***8-OH-2DG**, 8-Hydroxy-2'-deoxyguanosine; **ADMET,** Absorption, distribution, metabolism, excretion, and toxicity; **AHA**, Aromatic heavy atoms; **ANOVA,** One-way analysis of variance; **ATP**, Adenosine triphosphate; **ATPase**, Adenosine triphosphatase; **CDNB**, 1-Chloro-2,4-dinitrobenzene; **DNPH**, 2,4-Dinitrophenyl hydrazine; **EDTA**, Ethylenediaminetetraacetic acid; **GSH**, L-glutathione; **GST**, Glutathione S-transferase; **HA**, Heavy atoms; **HBA**, Hydrogen bond acceptor; **HBD**, Hydrogen bond donor; **HPLC**, High pressure liquid chromatography; **LD50**, Median lethal dose (a dose that proves lethal to 50% of a given population); **LogP**, Logarithm of the partition coefficient; **MDA**, Malondialdehyde; **MW**, Molecular weight; **PCC**, Protein carbonyl content; **PMSF**, Phenylmethanesulphonyl fluoride; **PSA**, Polar surface area; **RB**, Number of rotatable bonds; **ROS**, Reactive oxygen species; **SDA**, Sodium dodecyl sulfate; **SE**, Standard error; **SOD**, Superoxide dismutase; **TBA**, Thiobarbituric acid; **TCA**, Trichloroacetic acid; **β-NAD**, β-nicotinamide adenine dinucleotide.*

Abstract

The present study evaluated the adverse effects of three widely used insecticides in the Egyptian environment on mitochondrial bioenergetic and oxidative stress biomarkers in the rat kidney. Chlorpyrifos, cypermethrin, and imidacloprid were orally administrated to male albino rats at 1/50 of the LD50 for 28 days by five doses /week. The insecticides caused a significant in vivo decrease in the activities of mitochondrial bioenergetic ATPase and mitochondrial oxidative stress biomarkers, SOD, and GST, while MDA and PCC were significantly ($p \leq 0.05$) increased. Further, chromatography analysis demonstrated that 8-OH-2DG increased considerably in rat urine as a DNA damage biomarker. The kidney deficiency was confirmed by histological examination and in silico simulation analysis (molecular docking and ADMET). The alterations in the tested parameters were confirmed by the symptoms of histological deformation in kidney tissues, demonstrating the hazardous effects. The laboratory results showed the impact of the tested insecticides in conformity with the in silico simulation analysis.

Keywords: Oxidative stress; Rat kidney; Mitochondria; Insecticides; HPLC analysis; Molecular docking

Graphical Abstract



Introduction

As a primary excretory organ, the kidney is a major route of elimination for numerous xenobiotic agents to purify the blood by removing waste and harmful substances (Wei et al. 2021). Mitochondria are the main energy source in the cell and play a significant role in extensive oxidative metabolism and normal function (Wang et al. 2014). Mitochondria produce about 90% of the chemical energy cells need to survive and generate reactive oxygen species (ROS) that augment intracellular oxidative stress (Schofield and Schafer 2021). Therefore, the failure of mitochondrial ROS removal systems is believed to be the primary cause of intracellular oxidative stress.

Conversely, the role of mitochondrial ROS emission remains unknown, and a net increase in the production of ROS in mitochondria remains unclear (Boccatonda et al. 2016). Mitochondrial impairment can induce oxidative stress and reduce adenosine triphosphate (ATP) content (Samarghandian et al. 2015). These events are also closely linked to oxidative stress in the mitochondria. Na^+/K^+-ATPase failure can contribute to neuropathic pain in conditions of mitochondrial dysfunction, and ATP deficiency can result in chronic mitochondrial dysfunction (Lim et al. 2015).

Among the antioxidant enzymes, SOD plays a significant role in fighting free radical damage and inflammation (Uehara et al. 2021). This powerful enzyme forms the front line of defense against ROS that leads to cellular damage within the body. In addition, the mitochondrial glutathione system plays a crucial role in reducing H_2O_2 and protects mitochondria against peroxidative stress (Kotyk and Iskra 2021). GST is a Phase II detoxification enzyme that protects cellular macromolecules from attack by reactive electrophiles (Gao et al. 2021).

Protein carbonyl content (PCC) is the most commonly used biomarker of protein oxidation (Sharma et al. 2021). Because it is stable for long periods under proper storage conditions, it is advantageous in this respect. Several human diseases have accumulated PCC, including Alzheimer's, diabetes, inflammatory bowel disease, and arthritis (Sharma et al. 2020). In addition, malondialdehyde (MDA) is a highly reactive compound that forms covalent protein adducts with advanced lipoxidation end products, causing toxic stress in cells (Moldogazieva et al. 2019). This compound also forms mutagenic DNA adducts when it reacts with deoxyadenosine and deoxyguanosine in DNA.

In nuclear and mitochondrial DNA, 8-hydroxy-2'-deoxyguanosine (8-OH-2DG) is one of the predominant forms of accessible radical-induced oxidative lesions. Therefore, it has been widely used as a biomarker for oxidative stress and DNA damage (Mosa et al. 2019). Furthermore, in recent years, 8-OH-2DG has been used widely in many studies as a biomarker for measuring endogenous oxidative DNA damage and as a risk factor for many diseases, including cancer (Hinch et al. 2013).

Various pesticides can cause impaired energy regulation and cell dysfunction, and finally, cell death has been observed in many neurological disorders (De Castro et al. 2011). In pesticide-induced oxidative stress, pro-oxidant and antioxidant defense mechanisms are out of balance leading to oxidative stress (Deyashi and Chakraborty 2016). Consequently, pesticide intoxication alters antioxidant enzymes and glutathione redox systems in different tissues causing derangement of these mechanisms (Deyashi and Chakraborty 2016). For example, the insecticide chlorpyrifos induced apoptosis involved mitochondrial dysfunction through ROS production (Ahmed et al. 2010). Also, chlorpyrifos can cause oxidative stress and damage kidney structure (Ahmed et al. 2010). Apoptosis is also altered when other insecticides, including cypermethrin and imidacloprid, are exposed to oxidative stress and DNA damage.

Therefore, the present study determined the mitochondrial dysfunction and oxidative stress rat kidney following sub-chronic intoxication chlorpyrifos, cypermethrin, and imidacloprid. In addition, bioenergetic biomarkers, including NADH dehydrogenase and ATPase, were determined. Furthermore, oxidative stress biomarkers (PCC, MDA, and 8-OH-2DG) and antioxidant enzymes (SOD and GST) were measured. In addition, 8-OH-2DG levels as a biomarker of the DNA damage were measured by HPLC analysis in rat urine. In addition, histological analysis and *in silico* simulation analysis (molecular docking and ADMET) were studied in detail.

Materials and Methods

Insecticides and chemicals

Chlorpyrifos (96%), cypermethrin (96%), and imidacloprid (97%) were supplied from Zhejiang Rayfull Chemicals Co. (Zhejiang, China). Adenosine triphosphate (ATP), bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), 2,4- dinitrophenyl hydrazine (DNPH), ethylenediaminetetraacetic acid (EDTA), Folin-Ciocalteu′s phenol reagent, L-glutathione (GSH), β-nicotinamide adenine dinucleotide (β-NAD), phenylmethanesulphonyl fluoride (PMSF), sodium dodecyl sulfate (SDS), thiobarbituric acid (TBA), trichloroacetic acid (TCA), Tris (hydroxymethyl aminomethane), and 8-hydroxy-2'-deoxyguanosine (8-OH-2DG) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Animal treatment with insecticides

Fifty-day-old male albino rats weighing 120 ± 3 g were performed under the guidelines of the standard procedures established (OECD 2008). The institutional animal care and use committee (IACUC), Alexandria University, approved the experimental protocol (AU: 08181231101). According to the previous protocol, the animals were divided into four groups (5 animals/group) and orally treated five doses/week for 28 days (Taha et al. 2021). Group I: Rats administered with corn oil (1 ml/kg bw) were examined as a control. Group II: Rats were administered with chlorpyrifos 1.9 mg/kg bw (1/50 LD_{50}) (Tomlin 2004). Group III: Rats were administered with 5 mg/kg of cypermethrin (1/50 LD_{50}) (EPA 1989). Group IV:

Rats were administered with imidacloprid 9 mg/kg bw (1/50 LD_{50}) (Tomlin 1997).

Isolation of kidney mitochondria

Mitochondria from rat kidneys were isolated using a protocol of Krause et al. (2005) with some modifications as previously protocol (Taha et al. 2021).

Biochemical effects of the tested insecticides

Mitochondrial NADH dehydrogenase (Complex I) activity

NADH dehydrogenase activity was measured using NADH as a substrate (Galante and Hatefi 1978). Kidney mitochondria (40 µg of protein/ml) were mixed with a mixture containing 40 mM phosphate buffer, pH 7.4, 0.1% sodium cholate, and 1.3 mM potassium ferricyanide and incubated for 1 min at 30°C, then 0.14 mM NADH was added. The absorption decrease was measured spectrophotometrically at 340 nm using Unico 1200 Spectrophotometer (Laxco Inc, USA) for 1-3 minutes. Results were expressed as µmol NADH oxidized/min/mg protein. The protein content of the mitochondrial preparations was estimated by the Lowry et al. method (Lowry et al. 1951) using BSA as a standard.

Mitochondrial Mg-ATPase activity

The mitochondrial Mg-ATPase activity was estimated spectrophotometrically based on the formation of the inorganic phosphate (Pi) (Taussky and Shorr 1953) with minor modifications as described previously (Taha et al. 2021). The mitochondrial suspension (30 µl of 1 mg protein/ml) was added to a medium containing 20 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, and 5 mM ATP. Then the mixture was incubated at 37°C for 5 min with shaking. The addition of 5% TCA stopped the reaction, and then the inorganic phosphate (Pi) was measured spectrophotometrically at 740 nm. The data were expressed as µ mole Pi/mg protein.

Mitochondrial superoxide dismutase (SOD) activity

SOD was measured spectrophotometrically at 420 nm by pyrogallol as a substrate (Marklund and Marklund 1974) with some modifications (Taha et al. 2021). The assay medium was 1.0 ml containing 50 mM Tris-HCl buffer (pH 8.0) and 0.24 mM pyrogallol. Autoxidation of pyrogallol was monitored at 420 nm for 3 min in the absence and presence of enzyme at three concentrations, which produced between 30 to 60% inhibition of pyrogallol. The results were expressed as U/mg protein. One unit (U) of the enzyme activity is defined as the amount of enzyme, which produced 50% inhibition of pyrogallol autoxidation under the standard assay conditions.

Mitochondrial glutathione S-transferase (GST) activity

GST activity was measured by the simplified procedure of Vessey and Boyer (1984) using GSH and CDNB as substrates. First, the mitochondrial suspension (10 µL of 1 mg protein/mL) was added to a medium containing 0.1M phosphate buffer pH 6.5, 4mM GSH, and 1 mM CDNB. The mixture was incubated for 20 min at room temperature, and then the absorbance was measured at 340 nm. The activity was expressed as U/mg protein/ml using the $\mathcal{E}$ of CDNB 0.0096 $\mu M^{-1}\,cm^{-1}$.

Mitochondrial protein carbonyl content (PCC) level

The PCC was determined by the derivatization technique using DNPH as substrate (Reznick and Packer 1994). The PCC was determined by reading the absorbance at 375 nm of each sample against its appropriate blank. The data were expressed as nmol/mg protein/ml using the $\mathcal{E}$ of 22000 $M^{-1}\,cm^{-1}$.

Mitochondrial malondialdehyde (MDA) level

MDA was determined using TBA substrate according to Buege and Aust (1978). Briefly, 0.5 ml of mitochondrial suspension was reacted with 2 ml of TBA reagent containing 0.375% TBA, 15% TCA, and 0.25 N HCl. Samples will be boiled for 15 min, cooled, and centrifuged. The supernatant was measured at 535 nm. The TBA concentration was calculated using the $\mathcal{E}$ of 155 $mM^{-1}\,cm^{-1}$, and the results were expressed as nmol MDA/mg protein.

Urine 8-OH-2DG as a DNA biomarker

At the end of the experiment (4 weeks), urine samples were collected in a suitable tube for 8-OH-2DG determination (Prevost et al. 1990). Briefly, urine samples (2 ml) were diluted with an equal volume of 1 M NaCl and preconditioned the cartridge (C_{18} SPE cartridge) with 0.1 M KH_2PO_4, pH 7.5. The sample was loaded in the cartridge and washed with 5 ml of 50 mM KH_2PO_4 buffer (pH 7.5). Samples were eluted with 3 ml of 15% methanol in the same buffer then collected in the HPLC vials for

determination. The 8-OH-2DG was quantitively analyzed using an HPLC system, according to Taha et al. (2021) method.

Histological analysis

Histological analysis was done using Carleton's histological technique (Carleton et al. 1980). Single Kidney tissue was isolated from each control and insecticide-treated rat and then fixed in 10 % neutral buffered formalin for 24 h. The fixer was washed with running tap water overnight. After drying, the tissues were cleaned with methyl benzoate using a graded series of alcohols and embedded in wax with paraffin. At 4 mm thickness, kidney sections were cut, stained with hematoxylin (Drury and Wallington 1980), and eosin dissolved in 95% ethanol was used to stain the counter. Kidney sections were assembled by DPX and observed under a microscope (Leica Application Suite Version 4.12.0 (Build: 86), Wetzlar (Germany)) after dehydration and clearing.

Computational and *in silico* simulation analysis

ADMET prediction of the tested insecticides

The tested insecticides were submitted to an online *in silico* ADMET screening using the free website (http://www.swissadme.ch) to analyze their toxicity risks (Daina et al. 2017). The ADMET collection provides components that calculate predicted absorption, distribution, metabolism, excretion, and toxicity properties for groups of tested insecticides to assay the hazard effects of these compounds on the human body. Based on Lipinski's rule of five and its extensions (Lipinski et al. 1997), the molecular weight (MW), the logarithm of the partition coefficient (LogP), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), polar surface area (PSA), number of rotatable bonds (RB), heavy atoms (HA) and aromatic heavy atoms (AHA). The ADMET properties were predicted through aqueous solubility, blood-brain barrier, human intestinal absorption, permeability glycoprotein, and hepatotoxicity, evaluated for each insecticide within the human. In addition, AlogP98 and PSA_2D were used in plotting the confidence ellipses. The models used to predict the ADMET properties in this protocol are derived from various experimental data sources and are catalogued in the product documentation.

Molecular docking of vital mitochondrial enzymes

Molecular docking of the tested insecticides into NADH dehydrogenase, ATPase, SOD, and GST were performed. The crystal structures of ATPase (PDB: 2F43), NADH dehydrogenase (PDB: 6G2J), GST (PDB: 1GSC), and SOD (PDB: 1EM1) were obtained and retrieved from PDB at Brookhaven National Laboratory (http://www.rcsb.org/pdb/home/home.do). The structure of each enzyme was visualized by Molecular Operating Environment (MOE 2014.13) software (Chemical ComputingGroup 2008) and checked for missing atoms, bonds, and contacts, water molecules were removed, and polar hydrogen atoms were added. Then, the molecules were converted to a 3D structure. The Merck molecular force field (MMFF94) power was reduced with an iteration limit of 200 and the power threshold value of 15 kcal/mol above the minimum global energy until the local minimum energy was reached (Halgren 1999). The triangle-matching algorithm was selected from MOE for docking the compounds into the active sites of the desired protein. Free energy of binding was calculated from the contributions of hydrophobic, ionic, hydrogenated, and van der Waals interactions.

Statistical analysis

Data were statistically analyzed by IBM SPSS software version 25.0 (Statistical Package for Social Sciences, Chicago, IL, USA) (IBM 2017). All data were expressed as mean ± standard error (SE). Data were analyzed using one-way analysis of variance (ANOVA) followed by the Student–Newman-Keuls test to determine significance between different groups. The criterion for statistical significance was set at $p \leq 0.05$.

Results

Biochemical effects of the tested insecticide

Mitochondrial bioenergetic biomarkers

Table 1 presents the effects of the tested insecticides on NADH dehydrogenase and ATPase activity. The results show that ATPase activity recorded 15.30, 10.37, and 18.05 µmol Pi/mg protein/min for chlorpyrifos, cypermethrin, and imidacloprid, respectively compared to 36.16 in the control group. However, NADH dehydrogenase activity significantly ($p \leq 0.05$) decreased to 73.86, 47.62, and 56.43 nmol NADH oxidized/mg protein/min for chlorpyrifos, cypermethrin, and imidacloprid, respectively, compared to 117.68 in control. In general, the chemical treatment of rat kidneys inhibited

mitochondrial biomarkers by half. Cypermethrin had the greatest impact on ATPase inhibition, followed by chlorpyrifos and imidacloprid among the test compounds. Cypermethrin inhibited NADH dehydrogenase most strongly, followed by imidacloprid and chlorpyrifos.

Mitochondrial antioxidant enzymes

SOD activity of kidney mitochondria displayed significant reduction by 16.91, 7.68, and 11.38 U/mg protein for chlorpyrifos, cypermethrin, and imidacloprid, respectively, compared to 20.90 U/mg protein in the control group (**Table 2**). GST activity was also significantly ($p \leq 0.05$) declined to 8.00, 4.03, and 6.34 U/mg protein/ml for chlorpyrifos, cypermethrin, and imidacloprid, respectively, compared to control (10.47 U/mg protein/ml) (**Table 2**). Cypermethrin was more potent in SOD and GST reduction.

Mitochondrial oxidative stress biomarkers

The obtained results in **Table 2** indicated that the tested insecticides increased PCC by 0.15, 0.22, and 0.19 nmol/mg protein/ml for chlorpyrifos, cypermethrin, and imidacloprid, respectively, compared to 0.09 nmol/mg protein/ml in the control group. Cypermethrin oxidized mitochondrial proteins more effectively than imidacloprid and more effectively than chlorpyrifos. However, MDA levels were significantly ($p \leq 0.05$) increased to 2.22, 3.14 and 2.68 nmol/mg protein/ml for chlorpyrifos, cypermethrin and imidacloprid, respectively, compared to 1.48 nmol/mg protein/ml in control. Cypermethrin was more effective for oxidizing mitochondrial lipids, followed by imidacloprid and chlorpyrifos.

HPLC analysis of 8-OH-2DG as a DNA biomarker

Analysis conditions and recovery of 8-OH-2DG from rat urine

The 8-OH-2DG standard was scanned in the range of 230-345 nm against the mobile phase as a blank using a UV-Visible spectrophotometer. The maximum wavelength absorbance was found to be 252 nm. The calibration curve was linear up to ≥ 1.00 ug, and the correlation coefficient was =1.00. Regression equation analysis of the data (n = 5) for the calibration curve was y=4905.6x+0.0386. Recovery percentages of the 8-OH-2DG from rat urine at spiked levels of 5 and 10 µg/ml were 74.27% and 78.73%, respectively. Each value is the mean ± standard error of five replicate determinations.

Levels of 8-OH-2DG in rat urine

Chlorpyrifos, cypermethrin, and imidacloprid intoxication significantly ($p \leq 0.05$) increased 8-OH-2DG in urine compared to control (**Table 3**). Imidacloprid was the most active insecticide (11.32 µg 8-OH-2DG/ml) compared to 1.61 in the control group. As demonstrated in **Figure S1**, 8-OH-2DG chromatograms were measured in the rat groups of control, chlorpyrifos, cypermethrin, and imidacloprid, alongside a standard 8-OH-2DG and sample spiked at 5 g/ml (recovery).

Histopathological analysis

The kidney section of the control rat showed an intact histological structure of glomeruli and renal tubules. Cross-sections of kidneys showed many histological changes compared with those from the control rats. However, the changes of chlorpyrifos treated rats displayed hyperchromatic mesangial cells (MC), disturbed tubular epithelium (DT) and dilation in urinary space (DU). Hyper atrophied (HP) surrounded by inflammatory cells and proximal convoluted tubules (CT) with disturbed epithelial, necrosis (N), hemorrhage (Hm), damage and disorganization of kidney tubules (lost architecture) were also observed (**Figure 1**). Meanwhile, kidney sections of cypermethrin-treated rats were detected in glomeruli and convoluted tubules. The main characteristic findings were the appearance of; congested degenerative glomerular tuft, infiltration in between the degenerated tubules with fibrosis (F), swelling and rupture of the glomeruli (Rg), hemorrhage (Hm) in renal tubular and glomeruli, inflammatory cellular infiltration (Fi) between the renal tubules, and mild renal tubular and necrosis (N). The treatment with imidacloprid exhibited disturbed tubular epithelium (DT), dilation in urinary space (DU), hemorrhage (Hm), and necrotic (N). Atrophied (Ph) tubular epithelia were noticed in some individual glomeruli surrounded by inflammatory cells and congestion (G).

Table 1. *Kidney mitochondrial bioenergetics' biomarkers of male albino rats orally administrated with 1/50 of LD_{50} of chlorpyrifos, cypermethrin, and imidacloprid for 28 days (5 doses/week)*

Animal group	Dose (mg/kg bw)	Parameters (mean ± SE; n = 15)	
		ATPase (µmol Pi/mg protein/min)	NADH dehydrogenase (nmol NADH oxidized/mg protein/min)
Control	-	36.16^{a}±0.68	117.68^{a}±1.70
Chlorpyrifos	1.9	15.30^{c}±0.45	73.86^{b}±1.09
Cypermethrin	5.0	10.37^{d}±0.33	47.62^{d}±0.60
Imidacloprid	9.0	18.05^{b}±0.55	56.43^{c}±0.91

n is the number of replicates. Values in the column with different letters are significantly different at $p \leq 0.05$ using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls Test. ATPase: Adenosine triphosphatase. NADH dehydrogenase: Nicotinamide adenine dinucleotide dehydrogenase.

Table 2. *Kidney mitochondrial antioxidant enzymes and oxidative stress biomarkers of male albino rats orally administrated with 1/50 of LD_{50} of chlorpyrifos, cypermethrin, and imidacloprid for 28 days (5 doses/week)*

Animal group	Dose (mg/kg bw)	Parameters (mean ± SE; n = 15)			
		SOD (U/mg protein)	GST (U/mg protein/ml)	MDA (nmol/mg protein/ml)	PCC (nmol/mg protein/ml)
Control	-	20.90^{a}±1.45	10.47^{a}±0.66	1.48^{d}±0.03	0.09^{d}±0.004
Chlorpyrifos	1.9	16.91^{b}±1.10	8.00^{b}±0.59	2.22^{c}±0.04	0.15^{c}±0.007
Cypermethrin	5.0	7.68^{d}±0.45	4.03^{d}±0.25	3.14^{a}±0.07	0.22^{a}±0.010
Imidacloprid	9.0	11.38^{c}±0.54	6.34^{c}±0.31	2.68^{b}±0.05	0.19^{b}±0.008

n is the number of replicates. Values in the column with different letters are significantly different at $p \leq 0.05$ using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls Test. SOD: superoxide dismutase. GST: Glutathione S-transferase. MDA: Malondialdehyde. PCC: Protein carbonyl content.

Table 3. *Levels of 8-OH-2DG by HPLC in urine of male albino rats orally administrated with 1/50 of LD_{50} of chlorpyrifos, cypermethrin, and imidacloprid for 28 days (5 doses/week) measured*

Animal group	Dose (mg/kg bw)	(µg/ml)
Control	-	1.61^{d}±0.07
Chlorpyrifos	1.9	4.26^{c}±0.09
Cypermethrin	5.0	4.86^{b}±0.14
Imidacloprid	9.0	11.32^{a}±0.24

Values are mean of five replicates and given as mean ± standard error. Values in the column with different letters are significantly different at $p \leq 0.05$ using one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls Test.

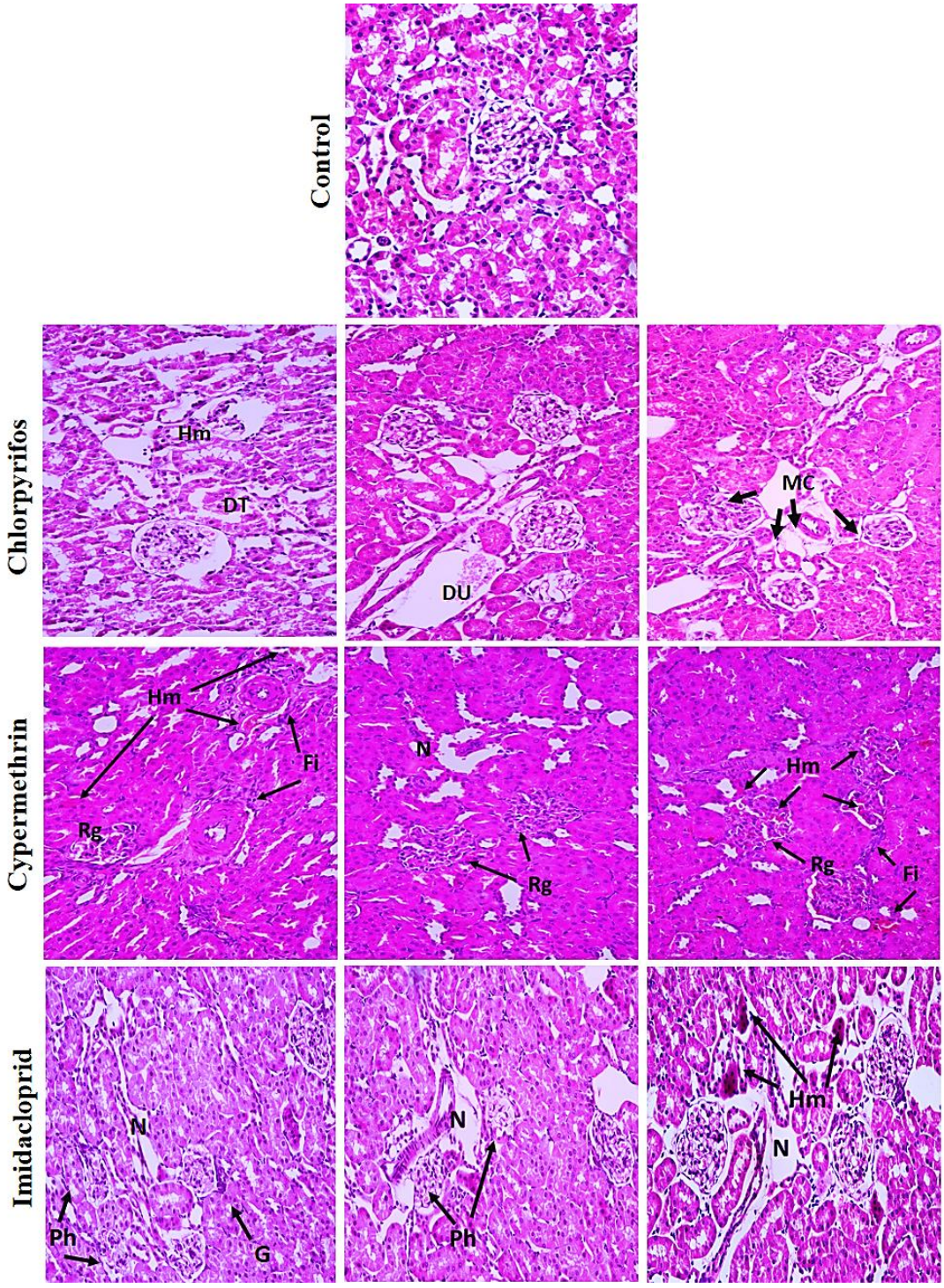


Figure 1. *Photomicrograph of the kidney sections of male rats orally dosed with chlorpyrifos (1.9 mg / Kg bw), cypermethrin (5 mg/kg bw) and imidacloprid (9 mg / Kg bw) which represented 1/50 of* LD_{50} *for 28 days (5 doses / week). Figure shows hyperchromatic mesangial cells (MC), disturbed tubular epithelium (DT), dilation in urinary space (DU) hyper atrophied (HP) surrounded by inflammatory cells, Necrosis (N), hemorrhage (Hm), Swelling and rupture of the glomeruli (Rg), inflammatory cellular infiltration (Fi) between the renal tubules, atrophied (Ph) surrounded by inflammatory cells and congestion(G) in the glomeruli.*

Computational and *in silico* simulation analysis

In silico ADMET prediction of the tested insecticides

The success of insecticides is determined by high efficacy against their target and an in-silico simulation to calculate and predict the ADMET (absorption, distribution, metabolism, excretion, and toxicity risks) profile (Moroy et al. 2012). The analysis of different terms such as LogP, MW, HBD, HBA, RB, and PSA of insecticides revealed that the insecticides are highly hydrophobic to penetrate the biological membranes according to the Lipinski "rule-of-five (**Table S1**). Extension of Lipinski rule of five includes the following criteria: number of rotatable bonds (RB) ≤10, topological polar surface area (PSA) ≤140 (Lipinski 2004). The tested insecticides had LogP in the range of 0.18 - 4.96 and PSA of 59.32 - 86.34, as shown in **Figure S2**. Therefore, according to the Lipinski rules, these results suggested that the insecticides have high toxicity on the biological system. Based on this analysis, it was reported that not all the insecticides showed any violations of Lipinski rules which confirm that these compounds are very high in absorption through human intestinal.

Molecular docking of insecticides with binding sites on target-enzymes

Docking on the NADH dehydrogenase

Docking results of insecticides on the NADH dehydrogenase (PDB ID: 6G2J) are listed in **Table 4**. The tested insecticides exhibited a high binding affinity towards the active sites of the enzyme. Cypermethrin showed the highest binding affinity with the lowest docking energy -3.11 kcal/mol. Followed by imidacloprid with docking energy -2.92 kcal/mol. However, chlorpyrifos was the lowest binding affinity on this enzyme with docking energy -2.34 kcal/mol (**Table 4**). **Figure 2A** shows the recognized binding modes and molecular orientations of cypermethrin. Through van der Waals interactions, six amino acids surround the insecticide (Asp 670, Gly 529, Ile 496, Met 673, Pro 664, and Thr 674). Moreover, it has interacted with four amino acids, Arg 495, Gln 665, Arg 675, and Arg 679 residues via HBA. The O1 atom in cypermethrin formed four HBA with the amino acids Arg 495, Gln 665, Arg 675, and Arg 679 residues via hydrogen bonding interaction (2.96, 3.42, 3.74, and 3.04 Å, respectively). However, the cyano group formed two HBA with the amino acids Arg 679 and Asp 675 via hydrogen bonding interaction (3.74 and 3.03 Å, respectively). In addition, there are three ionic interactions with the oxygen atom (Arg 495- O1, Arg 495- O1, and Arg 495- O28).

Docking on the ATPase

Docking results of the tested insecticides with the ATPase (PDB ID: 2F43) are shown in **Table 5**, and the interaction diagram is presented in **Figure 2B**. Cypermethrin showed the highest binding affinity with the lowest docking energy with docking energy -6.96 kcal/mol. Followed by chlorpyrifos with docking energy -5.05 kcal/mol. However, imidacloprid was the lowest binding affinity on this enzyme with docking energy -4.89 kcal/mol. All the investigated insecticides exhibited H-bonding interaction with amino acids in the active pockets of the enzyme. **Figure 2B** presented that the cypermethrin is surrounded by nine amino acids (Ala 401, Arg 398, Gln 405, Ile 361, Ile 365, Leu 356, Thr 425, Tyr 397, and Val 422) through van der Waals interactions. The O1 atom of the ester group formed HBA with the amino acid Lys 359 (3.33 Å). Moreover, one ionic interaction (Lys 359-O28), and one hydrophobic interaction (Leu 394-six-ring (Aryne)) with 3.13 and 4.44 Å, respectively, were observed.

Docking on the SOD

Docking results of chlorpyrifos, cypermethrin, and imidacloprid with SOD (PDB ID: 1EM1) are presented in **Table 6**. According to the results, the tested insecticides displayed a high binding affinity to the active site. Cypermethrin showed the highest binding affinity with the lowest docking energy -3.37 kcal/mol. Followed by imidacloprid with docking energy -2.86 kcal/mol. In contrast, chlorpyrifos was the lowest binding affinity on this enzyme with docking energy -2.13 kcal/mol. All insecticides showed H-bonding interactions with amino acids in the active pocket of the enzyme. Cypermethrin is surrounded by ten amino acids (Asn 84, Glu 88, Glu 187, Gly 85, Gly 91, Lys 90, Met 194, Pro 89, Thr 190, and Trp 186) through van der Waals interactions (**Figure 2C**). No H-bonds or hydrophobic interactions were observed. Two chlorine atoms formed two HBDs with Gly 86 and An 185.

Docking on the GST

Docking results of chlorpyrifos, cypermethrin, and imidacloprid with GST (PDB ID: 1GSC) are listed in **Table 7**. The data showed that the tested insecticides exhibited high binding affinity towards the active sites of the enzyme. Cypermethrin showed the highest binding

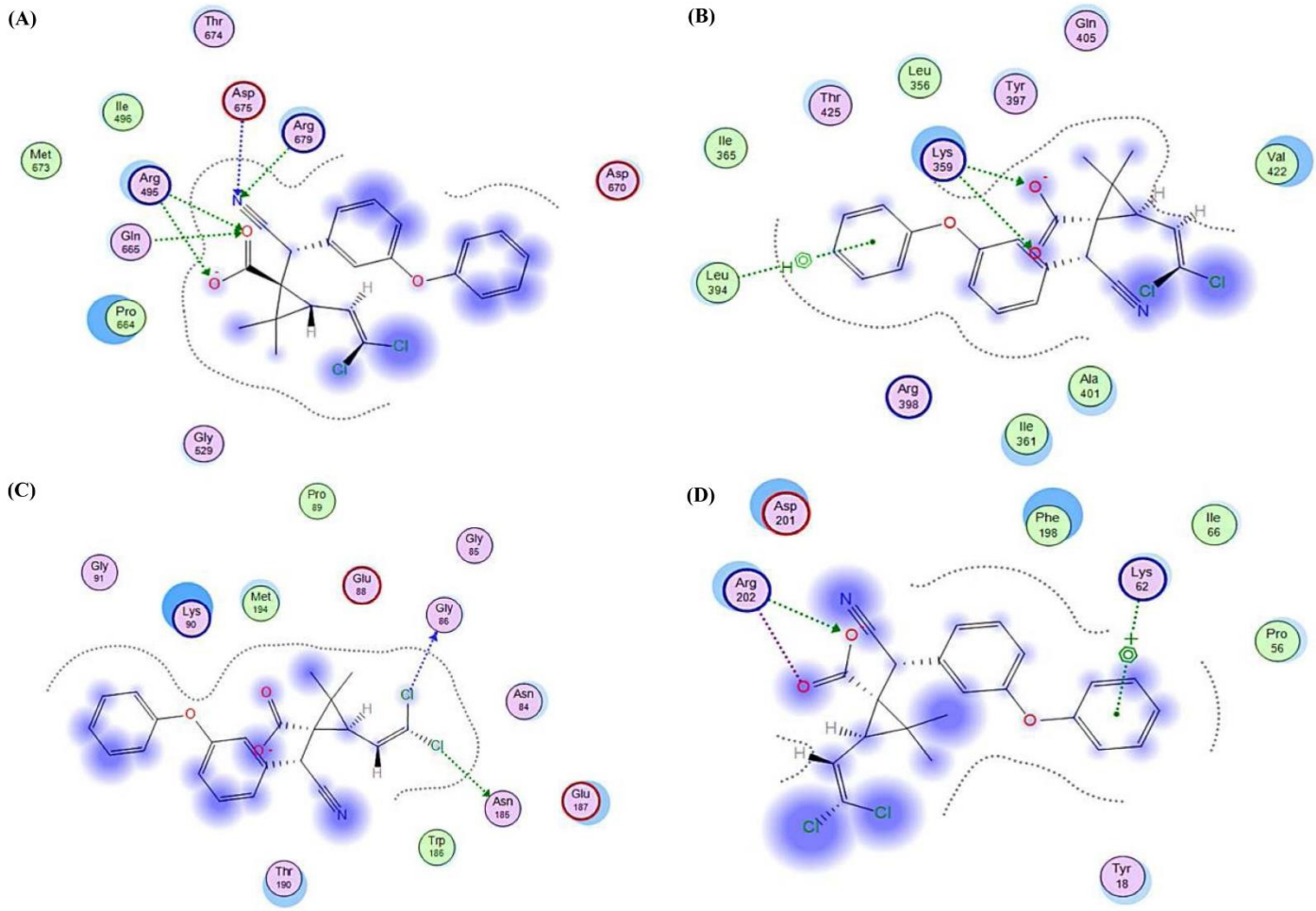


Figure 2. *Molecular docking of cypermethrin with binding sites of tested enzymes. A: NADH dehydrogenase (PDB ID: 6G2J). B: ATPase (PDB ID: 2F43). C: SOD (PDB ID: 1EM1). D: GST (PDB ID: 1GSC).*

affinity with the lowest docking energy (-8.71 kcal/mol). Imidacloprid followed with docking energy -5.06 kcal/mol (**Table 7**). At the same time, chlorpyrifos was the lowest binding affinity with docking energy of -4.89 kcal/mol. All insecticides exhibited H-bonding with amino acids in the active pocket of the enzyme. **Figure 2D** shows that the cypermethrin is surrounded by five amino acids (Asp 201, Ile 66, Phe 198, Pro 56, and Tyr 18) through van der Waals interactions. In addition, one amino acid, Arg 202 interacted via HBA with an oxygen atom (2.91 Å). In addition, this insecticide interacted with two amino acids (Arg 202 and Lys 62) through hydrophobic interactions with 2.91 and 4.03 Å, respectively.

Discussion

Biochemical effects of the tested insecticide

Mitochondrial bioenergetic biomarkers

In renal mitochondria, reduced energy production and increased oxidative damage are initial pathological events that direct acute kidney injury, tubular interstitial disease, cystic kidney disease, podocytopathy, and nephrotic syndromes (Bergman and Ben-Shachar 2016). The current study showed that the insecticides chlorpyrifos, cypermethrin, and imidacloprid caused mitochondrial dysfunction in rat kidneys. This finding was demonstrated by inhibiting NADH dehydrogenase

Table 4. Molecular docking, binding energy, binding scores and binding intereactions of chlorpyrifos, cypermethrin, and imidacloprid within the active sites of NADH (PDB ID: 6G2J)

Insecticides	Docking score (S) ΔG (kcal/mol)	Van der Waals	H-Bond			Hydrophobic Interactions (π-interactions)			RMSD
			(Amino acid-ligand atom)	Interaction	Distance (Å)	(Amino acid-ligand atom)	Interaction	Distance (Å)	
Chlorpyrifos	-2.35	Arg 495, Gln 459, Gln 665, Glu 346, Gly 529, Gly 530, Ile 496, Met 673, Pro 664	Thr 674-S1 Asp 675-S1 Arg 679-S1	HBA HBA HBA	4.14 4.33 4.47	-	-	-	3.007
Cypermethrin	-3.11	Asp 670, Gly 529, Ile 496, Met 673, Pro 664, Thr 674	Arg 495-O1 Gln 665-O1 Asp 675-N6 Arg 679-N6	HBA HBA HBA HBA	2.96 3.42 3.74 3.04	Arg 495- O1 Arg 495- O1 Arg 495- O28	Ionic Ionic Ionic	3.23 2.96 3.47	2.752
Imidacloprid	-2.92	Arg 679, Asp 528, Asp 675, Cys 531, Gln 665, Gly 529, Ile 496, Lys 266, Met 231	Asp 232-Cl14 Glu 346-N3	HBD HBD	4.10 2.76	Arg 495-6-ring Thr 674-6-ring	Aryne Aryne	4.14 3.68	1.405

RMSD: The root mean square deviation of the pose, in Å, from the original ligand. This field is present if the site definition was identical to the ligand definition.

Table 5. Molecular docking, binding scores and binding intereactions of chlorpyrifos, cypermethrin, and imidacloprid within the active sites of ATPase (PDB ID: 2F43)

Insecticides	Docking score (S) ΔG (kcal/mol)	Van der Waals	H-Bond			Hydrophobic Interactions (π-interactions)			RMSD
			(Amino acid-ligand atom)	Interaction	Distance (Å)	(Amino acid-ligand atom)	Interaction	Distance (Å)	
Chlorpyrifos	-5.05	Ala 364, Arg 398, Asn 366, Glu 353, Glu 355, Ile 361, Leu 36, Leu 394, Leu 428, Lys 429, Pro 363, Thr 425	Val 367-S1 Ile 365-N11	HBA HBA	4.32 3.30	-	-	-	1.607
Cypermethrin	-6.96	Ala 401, Arg398, Gln 405, Ile 361, Ile 365, Leu 356, Thr 425, Tyr 397, Val 422	Lys 359-O1	HBA	3.33	Lys 359-O28 Leu 394-6-ring	Ionic Aryne	3.13 4.44	2.828
Imidacloprid	-4.89	Ala 152, Ala 364, Arg 398, Asn 366, Ile 361, Leu 156, Leu 356, Leu 394, Pro 363, Thr 425	Val 367-O17	HBA	3.09	Ile 365-6-ring Leu 428- 6-ring	Aryne Aryne	4.56 3.83	1.096

RMSD: The root mean square deviation of the pose, in Å, from the original ligand. This field is present if the site definition was identical to the ligand definition.

(complex I) and Mg^{2+} ATPase. The inhibition may depend on the configurational structure of these enzymatic complexes. Since the complex I is a membrane-bound assembly of 45 different polypeptides (Carroll et al. 2006). While the ATPase is a multi-component structure that spans the inner membrane of mitochondria, the cell's energy generators (Mühleip et al. 2019). Numerous pesticides have been reported to impair mitochondrial function via different mechanisms and dysfunction of this organelle (Guven et al. 2018). For example, chlorpyrifos and dichlorvos produced oxidative stress and neurotoxicity by inhibiting NADH dehydrogenase activity (Binukumar et al. 2010). Complex I is also inhibited by permethrin and cyhalothrin in isolated rat liver mitochondria; this may be related to ROS production (Guven et al. 2018). Indeed, the lack of NADH oxidation strongly decreases NAD^+ levels, thus obstructing the activity of the different NAD^+-dependent dehydrogenases of the β-oxidation and tricarboxylic acid cycle pathways (Massart et al. 2018). Deficits in this cycle can cause hyperlactatemia and lactic acidosis, as lactate dehydrogenase converts pyruvate into lactate when NADH levels are excessive (Margolis et al.

Table 6. Molecular docking, binding scores and binding intereactions of chlorpyrifos, cypermethrin, and imidacloprid within the active sites of SOD (PDB ID: 1EM1)

Insecticides	Docking score (S) ΔG (kcal/mol)	Van der Waals	H-Bond			Hydrophobic Interactions (π-interactions)			RMSD
			(Amino acid-ligand atom)	Interaction	Distance (Å)	(Amino acid-ligand atom)	Interaction	Distance (Å)	
Chlorpyrifos	-2.13	Asn 84, Asn 185, Gly 86, Gly 87, Pro 89, Trp 186	Gly 85-S1 Glu 187-S1 Glu 88-Cl	HBA HBA HBD	4.41 3.94 4.12	-	-	-	1.454
Cypermethrin	-3.37	Asn 84, Glu 88, Glu 187, Gly 85, Gly 91, Lys 90, Met 194, Pro 89, Thr 190, Trp 186	Gly 85-Cl1 Asn 185-Cl2	HBD HBD	4.68 4.34	-	-	-	1.396
Imidacloprid	-2.87	Asn 185, Glu 88, Gly 85, Gly 86, Ile 184, Lys 90, Pro 89, Thr 190, Trp 186	Asn 84-N3 Glu 187-O17	HBD HBA	2.99 3.08	-	-	-	1.343

RMSD: The root mean square deviation of the pose, in Å, from the original ligand. This field is present if the site definition was identical to the ligand definition.

Table 7. Molecular docking, binding scores and binding intereactions of chlorpyrifos, cypermethrin, and imidacloprid within the active sites of GST (PDB ID: 1R4W)

Insecticides	Docking score (S) ΔG (kcal/mol)	Van der Waals	H-Bond			Hydrophobic Interactions (π-interactions)			RMSD
			(Amino acid-ligand atom)	Interaction	Distance (Å)	(Amino acid-ligand atom)	Interaction	Distance (Å)	
Chlorpyrifos	-4.89	Asn 53, Gly 182, Leu 183, Phe 181, Phe 198	Pro 56-S1 Lys 62-S1 Met 48-Cl	HBA HBA HBA	4.19 4.12 4.68	-	-	-	2.002
Cypermethrin	-8.71	Asp 201, Ile 66, Phe 198, Pro 56, Tyr 18	Arg 202-O1	HBA	2.91	Arg 202-O28 Lys 62-6-ring	Ionic Aryne	2.91 4.03	1.802
Imidacloprid	-5.06	Gly 182, Ile 66, Leu 44, Phe 87, Phe 88, Phe 198, Pro 56, Ser 16, Tyr 18	Met 48-N3 Lys 62-CL14 Leu 183-O17	HBD HBA HBA	4.23 3.65 3.34	-	-	-	1.101

RMSD: The root mean square deviation of the pose, in Å, from the original ligand. This field is present if the site definition was identical to the ligand definition.

2014). In a study by Muhammad et al., cypermethrin at 1/10 and 1/30 LD_{50} caused a significant decrease in NADH dehydrogenase and ATPase from brain mitochondria of adult male albino rats (Muhammed et al. 2020). Consistent with our observations, Mota et al. (2011) found that organochlorine pesticides decreased mitochondria number and ATP levels in treated rats. According to the study of Abdel-Razik (2019), imidacloprid significantly reduced brain mitochondrial NADH dehydrogenase and ATPase activities. Also, Arellano-Carrillo et al. (2017) reported that deltamethrin modified plasma membrane Ca^{2+}-ATPase levels sampled at different time points in human lymphocytes due to long-term exposure. Our results clearly show that the three insecticides inhibited the mitochondrial respiratory enzymes NADH dehydrogenase (complex I) and ATPase (complex V). As a result, they may cause a redox imbalance or impair mitochondrial membrane potential, resulting in mitochondrial enzyme structure degradation.

Mitochondrial antioxidant enzymes

GST and SOD are well known for their role in protecting cells from chemically induced cytotoxicity (Thowfeik 2016). In the present study, SOD and GST

activities were significantly ($p \leq 0.05$) decreased in the kidney after treating with selected insecticides. This result agrees with Abdel-Razik (2019), who reported that imidacloprid reduced the brain mitochondria SOD activity, while the GST activity was increased in treated mice. Also, Abdel-Daim and Abdeen (2018) revealed that fipronil significantly decreased the rat liver and kidney levels of CAT, SOD, and GPx. In addition, long-term exposure to OPs and carbamates may reduce the antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPX), and GST or stimulate the production of antioxidants (Zafiropoulos et al. 2014). Abbassy et al. reported a significant reduction in GST activity in plasma of male rats after lambda-cyhalothrin administration (Abbassy et al. 2014). A similar significant decrease in GST activity was observed in rat liver treated with chlorpyrifos (Mansour and Mossa 2010). This data suggests that SOD and GST play an essential role in balancing mitochondria redox events.

Mitochondrial oxidative stress biomarkers

Mitochondria are a significant generator of ROS, which cause damage to the subcellular target proteins, lipids, and DNA in the absence of proper protection (Thowfeik 2016). Carbonyl groups can be induced by almost all ROS and lipid peroxidation products, such as MDA. Dalle-Donne et al. (2003) mentioned that the relative early production and stability of carbonylated proteins could suggest chronic oxidation. Lipid peroxidation can change the membrane permeability or disrupt calcium homeostasis (Pena-Bautista et al. 2019).

In agreement with the obtained data, Abdel-Daim and Abdeen (2018) revealed that the insecticide fipronil significantly increased the liver and kidney contents of MDA and nitric oxide in treated rats. In addition, Abdel-Razik (2019) reported that imidacloprid dosed mice displayed a significant increase in brain mitochondria LPO and PCC levels. Moreover, Kubrak et al. (2012) recorded an increase in the PCC in blood lipid peroxide in gills after exposure to 10 mg/L of mancozeb. Also, Goswami et al. (2020) found that chloropicrin increased PCC in human corneal epithelial cells. MDA levels in rat liver and kidney tissues were also significantly increased by cypermethrin and methyl parathion (Gomaa et al. 2011). Bifenthrin has the affinity to damage cellular proteins and increased PCC in the selected brain regions (Syed et al. 2018).

8-OH-2DG as a DNA biomarker

Increased oxidative stress or disease states have been associated with 8-OH-2DG, a repair product of oxidized guanine lesions. It can be used as a reliable biomarker of oxidative DNA and RNA damage and repair (Taha et al. 2021). The authors found a linear link between ROS production and the formation of 8-OH-2DG, implying that ROS can trigger 8-OH-2DG formation. Pesticides may play a key role in producing oxidative stress, according to data on the increased risk of high 8-OH-2DG among farmers exposed to various kinds of pesticides (Jelić et al. 2018). Muniz et al. (2008) found 2.3 times higher urine 8-OH-2DG concentrations in sprayers and 8.5 times higher concentrations in agricultural laborers in a sample of 31 people. Similarly, pesticide sprayers who had been exposed to OP insecticides for a long time had higher levels of 8-OH-2DG (Lee et al. 2017). Also, Umemura et al. (2000) reported no increased concentration of 8-OH-2DG levels in the kidney nuclear DNA following sub-chronic exposure of rats to *p*-dichlorobenzene. Another study discovered that greater urine levels 8-OH-2DG were linked to increased exposure to OP insecticides (Ding et al. 2012). On the other hand, Tope and Panemangalore (2007) observed no changes in urine 8-OH-2DG levels between pesticide sprayers and the control group. Still, they identified higher levels of the same marker in the plasma of the pesticide sprayers.

Histopathological assessment of renal tissues

ROS-derived damage to natural and structured cellular components is generally considered a severe mechanism involved in histological disorders (Sepici-Dinçel et al. 2009). Chlorpyrifos may reduce regeneration of necrotic tissue by methylating and phosphorylating cellular proteins (Murray et al. 2003). The obtained results revealed that chlorpyrifos caused many abnormalities in kidney sections. This finding is consistent with studies that have indicated certain OP pesticides, including chlorpyrifos, were capable of causing kidney damage in rats, including marked tubular dilation, hydropic degeneration of the epithelium in the tubular lining, moderate congestion, and hemorrhage (Afshar et al. 2008; Kerem et al. 2007). Similarly, Heikal et al. (2012) recognized that the renal histoarchitecture of the chlorpyrifos, cyromazine, and chlorpyrifos + cyromazine treated rats showed swelling endothelium glomerular tuft, swelling in the lining epithelium of tubules, and

inflammatory cells infiltration in between the degenerated tubules.

According to the current histological analysis, cypermethrin intoxication adversely affected the kidney tissues of the treated animal. Hemorrhage was the essential symptom of distinctive cypermethrin poisoning, resulting from increased pressure within the portal vein redirecting to portal hypertension. Marrs (2012) revealed histological changes in kidney tissues of rats treated with cypermethrin. Prashanth (2011) reported that cypermethrin at lethal and sublethal concentrations caused considerable histological damage to the kidney tissues of fish. Grewal et al. (2010) reported that cypermethrin intoxication resulting deleterious impact in the form of shrinkage of glomeruli, necrosis of renal tubules, hemorrhage, and sloughing off renal epithelial cells in the convoluted tubules in kidney tissues. Also, imidacloprid caused different histological changes in the kidney sections. Similar histopathological injuries were found in kidneys of Japanese quail exposed to imidacloprid for six weeks (Eissa 2004) and in layer chickens exposed to 139 mg/kg imidacloprid (Kammon et al. 2010). Likewise, previous studies have shown that imidacloprid exposure leads to pathological deviations and genotoxic effects in the non-target organisms such as fish and rabbits (Stivaktakis et al. 2014).

Computational and *in silico* simulation analysis

Possible associations between different pesticides and specific targets are proposed and well established based on new *in silico* studies (Badawy et al. 2021; Villaverde et al. 2017). Today, it is recognized that employing computational chemistry as early as possible in the drug discovery process helps to reduce the number of safety issues (Badawy et al. 2019). The ADMET properties of the tested insecticides are of vital importance in this study to support the results obtained. The ADMET analysis confirmed that all insecticides achieved the descriptor criteria at the optimal level (Lipinski et al. 1997).

The industry of research-based pesticides increasingly uses modern medical chemistry methods, including molecular docking (Hughes et al. 2011). Molecular docking is the method used to analyze the positioning or orientation of tested molecules on their potential targets to predict the binding affinity and interactions. Once a compound (ligand)-enzyme complex has been determined, the biological activity data are correlated to the structural information (Shoichet and Kobilka 2012). Hence, the present research hypothesis compares three insecticides with NADH, ATPase, SOD, and GST. The data showed different interactions, including van der Waals, H-bonds, and H-pi hydrophobic. These interactions have been used to elucidate several biologically active compounds in diverse areas, such as pharmaceutics, pesticides and antimicrobials (Badawy et al. 2021). Many scientific reports have claimed that the most active biologically compounds as enzyme inhibitors contain these types of interactions with the target receptors (Badawy et al. 2021; Taktak et al. 2021). Typically, hydrophobic inhibitors are bonded with their molecular targets with high affinity, leading to a long-term inhibition response (Taylor et al. 2002). This concept led to attention to insecticide interaction with target proteins, predicting which one is more toxic. Knowledge gained from such investigations may be employed to develop more potent, selective, and efficient analogs (Badawy 2020).

Ethics approval and consent to participate

The institutional animal care and use committee (IACUC), Alexandria University, with reference number, approved the experimental protocol on December 31, 2018 (AU: 08181231101). The study was carried out in compliance with the International Guidelines for Research Ethics.

Declaration of competing interest

All the authors confirm that the content of this article has no conflict of interest.

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Availability of data and materials

All data generated or analyzed during this study are included in this article. Also, the related datasets are available from the corresponding author on reasonable request.

Authors' contributions

All authors contributed to the study conception and design. They performed material preparation, data collection, and analysis. Mostafa A. I. Taha performed toxicological and biochemical studies on male albino rats.

Mohamed E. I. Badawy performed the HPLC analysis of 8-OH-2-DG biomarker in rat urine samples and computer simulation analyses. Reda K. Abdel-Razik performed the histological analysis of the experiments. Mahmoud M. Abo-El-Saad and Hassan M. Younis supervised all the experiments and revised the data and the manuscript. All authors participated in manuscript writing, proofreading, sentence correction and approved the final manuscript.

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Not applicable

References

Abbassy MA, Marzouk MA, Mansour SA, Shaldam HA, Mossa AH. (2014). Impact of oxidative stress and lipid peroxidation induced by lambdacyhalothrin on p450 in male rats: the ameliorating effect of zinc. Environ Anal Toxicol. 4: 1-5.

Abdel-Daim MM, Abdeen A. (2018). Protective effects of rosuvastatin and vitamin E against fipronil-mediated oxidative damage and apoptosis in rat liver and kidney. Food Chem Toxicol. 114: 69-77.

Abdel-Razik RK. (2019). The protective effect of *Nigella sativa* oil on neurodisorder and oxidative stress driven by imidacloprid in mice mitochondria. Eg Sci J Pestic. 5: 22-31.

Afshar S, Farshid AA, Heidari R, Ilkhanipour M. (2008). Histopathological changes in the liver and kidney tissues of Wistar albino rat exposed to fenitrothion. Toxicol Ind Health. 24: 581-586.

Ahmed NS, Mohamed AS, Abdel-Wahhab MA. (2010). Chlorpyrifos-induced oxidative stress and histological changes in retinas and kidney in rats: Protective role of ascorbic acid and alpha tocopherol. Pestic Biochem Physiol. 98: 33-38.

Arellano-Carrillo MD, Vargas-Medrano J, Bojorquez-Rangel G, Perez-Leon J, Plenge-Tellechea LF. (2017). Long term exposure to deltamethrin causes a dual effect on plasma membrane Ca 2+-ATPase activity and reduces its mRNA levels in human lymphocytes. Indian J Exp Biol. 55: 271-278.

Badawy MEI (2020) Pharmacophore modeling and virtual screening for the discovery of biologically active natural products. In: Studies in Natural Products Chemistry, vol 64. Elsevier, pp 321-364

Badawy MEI, Abd-Elnabi AD, Saad A-FSA. (2021). Insecticidal activity of nanoemulsions of organophosphorus insecticides against cotton leafworm (*Spodoptera littoralis*) and molecular docking studies. Int J Trop Insect Sci. 1-21.

Badawy MEI, Marei GIKh, Rabea EI, Taktak NEM. (2019). Antimicrobial and antioxidant activities of hydrocarbon and oxygenated monoterpenes against some foodborne pathogens through in vitro and in silico studies. Pestic Biochem Physiol. 158: 185-200.

Bergman O, Ben-Shachar D. (2016). Mitochondrial oxidative phosphorylation system (OXPHOS) deficits in schizophrenia: possible interactions with cellular processes. The Canad J Psyc. 61: 457-469.

Binukumar BK, Bal A, Kandimalla R, Sunkaria A, Gill KD. (2010). Mitochondrial energy metabolism impairment and liver dysfunction following chronic exposure to dichlorvos. Toxicology. 270: 77-84.

Boccatonda A, Tripaldi R, Davì G, Santilli F. (2016). Oxidative stress modulation through habitual physical activity. Curr Pharm Des. 22: 3648-3680.

Buege JA, Aust SD. (1978). Microsomal lipid peroxidation Methods in Enzymology. 52: 302-310.

Carleton HM, Drury RAB, Wallington EA (1980) Carleton's histological technique. Oxford University Press, USA,

Carroll J, Fearnley IM, Skehel JM, Shannon RJ, Hirst J, Walker JE. (2006). Bovine complex I is a complex of 45 different subunits. J Biol Chem. 281: 32724-32727.

ChemicalComputingGroup M (2008) Molecular Operating Environment, 2008.10. Montreal,

Daina A, Michielin O, Zoete V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 7: 42717.

Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. (2003). Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta. 329: 23-38.

De Castro IP, Martins LM, Loh SHY. (2011). Mitochondrial quality control and Parkinson's disease: a pathway unfolds. Mol Neurobiol. 43: 80-86.

Deyashi M, Chakraborty SB. (2016). Pesticide induced oxidative stress and the role of antioxidant defense system in animal body. Harvest. 2: 1-14.

Ding G, Han S, Wang P, Gao Y, Shi R, Wang G, Tian Y. (2012). Increased levels of 8-hydroxy-2′-deoxyguanosine are attributable to organophosphate pesticide exposure among young children. Environ Pollut. 167: 110-114.

Drury RAB, Wallington EA (1980) Light microscope and slide preparation. Carleton's histological technique. Oxford University Press, London,

Eissa OS. (2004). Protective effect of vitamin C and glutathione against the histopathological changes induced by imidacloprid in the liver and testis of Japanese quail. The Eg J Hosp Med. 16: 39-54.

EPA U. (1989). Cypermethrin Pesticide Fact Sheet. US Environmental Protection Agency, Office of Pesticide Programs, Registration Div, Washington, DC. Number 199.:

Galante YM, Hatefi Y (1978) [4] Resolution of complex I and isolation of NADH dehydrogenase and an iron-sulfur protein. In: Methods in Enzymol, vol 53. Elsevier, pp 15-21

Gao H, Lin X, Yang B, Liu Z. (2021). The roles of GSTs in fipronil resistance in Nilaparvata lugens: Over-expression and expression induction. Pestic Biochem Physiol. 104880.

Gomaa M, Abd Alla M, Sameer MM. (2011). The possible protective effect of propolis (Bee glue) on cypermethrin-induced hepatotoxicity in adult albino rats. Mansoura J Forensic Med Clin Toxicology. 19: 17-32.

Goswami DG, Kant R, Ammar DA, Agarwal C, Gomez J, Agarwal R, Saba LM, Fritz KS, Tewari-Singh N. (2020). Toxic consequences and oxidative protein carbonylation from chloropicrin exposure in human corneal epithelial cells. Toxicol Lett. 322: 1-11.

Grewal KK, Sandhu GS, Kaur R, Brar RS, Sandhu HS. (2010). Toxic impacts of cypermethrin on behavior and histology of certain tissues of albino rats. Toxicol Int. 17: 94-98.

Guven C, Sevgiler Y, Taskin E. (2018). Pyrethroid insecticides as the mitochondrial dysfunction inducers. Mitochon Dis. 293-322.

Halgren TA. (1999). MMFF VI. MMFF94s option for energy minimization studies. J Comput Chem. 20: 720-729.

Heikal TM, Mossa ATH, Marei GIK, Abdel Rasoul MA. (2012). Cyromazine and chlorpyrifos induced renal toxicity in rats: the ameliorating effects of green tea extract. Environ Analyt Toxicol. 2: 2161.

Hinch EC, Sullivan-Gunn MJ, Vaughan VC, McGlynn MA, Lewandowski PA. (2013). Disruption of pro-oxidant and antioxidant systems with elevated expression of the ubiquitin proteosome system in the cachectic heart muscle of nude mice. Journal of Cachexia, Sarcopenia and Muscle. 4: 287-293.

Hughes JP, Rees S, Kalindjian SB, Philpott KL. (2011). Principles of early drug discovery. The British Journal of Pharmacology. 162: 1239-1249.

IBM. (2017). Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. . Armonk, NY: IBM Corp

Jelić M, Mandić A, Kladar N, Sudji J, Božin B, Srdjenović B. (2018). Lipid peroxidation, antioxidative defense and level of 8-hydroxy-2-deoxyguanosine in cervical cancer patients. J Med Biochem. 37: 336.

Kammon AM, Brar RS, Banga HS, Sodhi S. (2010). Patho-biochemical studies on hepatotoxicity and nephrotoxicity on exposure to chlorpyrifos and imidacloprid in layer chickens. Veterinarski Arhiv. 80: 663-672.

Kerem M, Bedirli N, GürbüZ N, Ekinci O, Bedirli A, Akkaya T, Şakrak Ö, PAŞAOĞLU H. (2007). Effects of acute fenthion toxicity on liver and kidney function and histology in rats. Turk J Med Sci. 37: 281-288.

Kotyk B, Iskra R. (2021). Effects of ethylthiosulfanylate and chromium (VI) on the state of glutathione antioxidant system and oxidative stress marker content in rat kidneys. Curr Appl Sci Technol. 761-773.

Krause F, Reifschneider NH, Goto S, Dencher NA. (2005). Active oligomeric ATP synthases in mammalian mitochondria. Biochem Biophys Res Commun. 329: 583-590.

Kubrak OI, Atamaniuk TM, Husak VV, Drohomyretska IZ, Storey JM, Storey KB, Lushchak VI. (2012). Oxidative stress responses in blood and gills of Carassius auratus exposed to the mancozeb-containing carbamate fungicide Tattoo. Ecotoxicol Environ Saf. 85: 37-43.

Lee KM, Park S-Y, Lee K, Oh S-S, Ko SB. (2017). Pesticide metabolite and oxidative stress in male farmers exposed to pesticide. Ann Occup Environ Med. 29: 1-7.

Lim TKY, Shi XQ, Johnson JM, Rone MB, Antel JP, David S, Zhang J. (2015). Peripheral nerve injury induces persistent vascular dysfunction and endoneurial hypoxia, contributing to the genesis of neuropathic pain. J Neurosci. 35: 3346-3359.

Lipinski CA. (2004). Lead- and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies. 1: 337-341.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Del Rev. 23: 3-25.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with the Folin phenol reagent. J Biol Chem. 193: 265-275.

Mansour SA, Mossa A-TH. (2010). Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pestic Biochem Physiol. 96: 14-23.

Margolis AM, Heverling H, Pham PA, Stolbach A. (2014). A review of the toxicity of HIV medications. J Med Toxicol. 10: 26-39.

Marklund S, Marklund G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. The Eur J Biochem. 47: 469-474.

Marrs TC (2012) Mammalian toxicology of insecticides. vol 12. Royal Soci Chem.

Massart J, Borgne-Sanchez A, Fromenty B (2018) Drug-induced mitochondrial toxicity. In: Mitochondrial biology and experimental therapeutics. Springer, pp 269-295

Moldogazieva NT, Mokhosoev IM, Mel'nikova TI, Porozov YB, Terentiev AA. (2019). Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases. Oxid Med Cell Longev. 2019: 3085756.

Moroy G, Martiny VY, Vayer P, Villoutreix BO, Miteva MA. (2012). Toward in silico structure-based ADMET prediction in drug discovery. Drug Discov Today. 17: 44-55.

Mosa IF, Yousef MI, Kamel M, Mosa OF, Helmy Y. (2019). The protective role of CsNPs and CurNPs against DNA damage, oxidative stress, and histopathological and immunohistochemical alterations induced by hydroxyapatite nanoparticles in male rat kidney. Toxicol Res. 8: 741-753.

Mota PC, Cordeiro M, Pereira SP, Oliveira PJ, Moreno AJ, Ramalho-Santos J. (2011). Differential effects of p, p′-DDE on testis and liver mitochondria: Implications for reproductive toxicology. Reprod Toxicol. 31: 80-85.

Muhammed RE, El-Desouky MA, Abo-Seda SB, Nahas AA, Elhakim HKA, Alkhalaf MI. (2020). The protecting role of *Moringa oleifera* in cypermethrin-induced mitochondrial dysfunction and apoptotic events in rats brain. Journal of King Saud University-Science. 32: 2717-2722.

Mühleip A, McComas SE, Amunts A. (2019). Structure of a mitochondrial ATP synthase with bound native cardiolipin. Elife. 8: e51179.

Muniz JF, McCauley L, Scherer J, Lasarev M, Koshy M, Kow Y, Nazar-Stewart V, Kisby G. (2008). Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. Toxicol Appl Pharmacol. 227: 97-107.

Murray L, Cooper PJ, Wilson A, Romaniuk H. (2003). Controlled trial of the short-and long-term effect of psychological treatment of post-partum depression: 2. Impact on the mother-child relationship and child outcome. The British J Psyc. 182: 420-427.

OECD. (2008). Guidance document on acute oral toxicity for the testing of chemicals. Environ Health Saf Monog Series on Testing and Assess. 1-27.

Pena-Bautista C, Vento M, Baquero M, Chafer-Pericas C. (2019). Lipid peroxidation in neurodegeneration. Clin Chim Acta. 497: 178-188.

Prashanth MS. (2011). Histopathological changes observed in the kidney of freshwater fish, Cirrhinus mrigala (Hamilton) exposed to cypermethrin. Rec Res Sci Technol. 3: 59-65.

Prevost V, Shuker DEG, Bartsch H, Pastorelli R, Stillwell WG, Trudel LJ, Tannenbaum SR. (1990). The determination of urinary 3-methyladenine by immnunoaffinity chromatography-monoclonal antibody-based ELISA: use in human biomonitoring studies. Carcinogenesis. 11: 1747-1751.

Reznick AZ, Packer L. (1994). Oxidative damage to proteins: spectrophotometric method for carbonyl Method in Enzymol. 233: 357-363.

Samarghandian S, Azimi-Nezhad M, Shabestari MM, Azad FJ, Farkhondeh T, Bafandeh F. (2015). Effect of chronic exposure to cadmium on serum lipid, lipoprotein and oxidative stress indices in male rats. Interdiscip Toxicol. 8: 151.

Schofield JH, Schafer ZT. (2021). Mitochondrial reactive oxygen species and mitophagy: a complex and nuanced relationship. Antioxid Redox Signal. 34: 517-530.

Sepici-Dinçel A, Benli AÇK, Selvi M, Sarıkaya R, Şahin D, Özkul IA, Erkoç F. (2009). Sublethal cyfluthrin toxicity to carp (*Cyprinus carpio* L.) fingerlings: biochemical, hematological, histopathological alterations. Ecotoxicol Environ Saf. 72: 1433-1439.

Sharma A, Sharma M, Rawat S, Mittal A, Kumar S. (2021). Correlation of glycated haemoglobin with protein carbonyl content as biomarkers of oxidative stress in Type 2 diabetes mellitus. Nat J Lab Med. 2: BO09 - BO11.

Sharma A, Weber D, Raupbach J, Dakal TC, Fliessbach K, Ramirez A, Grune T, Wullner U. (2020). Advanced glycation end products and protein carbonyl levels in plasma reveal sex-specific differences in Parkinson's and Alzheimer's disease. Redox Biology. 34: 101546.

Shoichet BK, Kobilka BK. (2012). Structure-based drug screening for G-protein-coupled receptors. Trends Pharmacol Sci. 33: 268-272.

Stivaktakis P, Kavvalakis M, Goutzourelas N, Stagos D, Tzatzarakis M, Kyriakakis M, Rezaee R, Kouretas D, Hayes W, Tsatsakis A. (2014). Evaluation of oxidative stress in long-term exposed rabbits to subtoxic levels of imidacloprid. Toxicol Lett. 229: S228.

Syed F, Awasthi KK, Chandravanshi LP, Verma R, Rajawat NK, Khanna VK, John PJ, Soni I. (2018). Bifenthrin-induced neurotoxicity in rats: involvement of oxidative stress. Toxicol Res. 7: 48-58.

Taha MAI, Badawy MEI, Abdel-Razik RK, Younis HM, Abo-El-Saad MM. (2021). Mitochondrial dysfunction and oxidative stress in liver of male albino rats after exposing to sub-chronic intoxication of chlorpyrifos, cypermethrin, and imidacloprid. Pestic Biochem Physiol. 178: 104938.

Taktak NEM, Badawy MEI, Awad OM, Abou El-Ela NE, Abdallah SM. (2021). Enhanced mosquitocidal efficacy of pyrethroid insecticides by nanometric emulsion preparation towards Culex pipiens larvae with biochemical and molecular docking studies. J Egypt Public Health Assoc. 96: 1-19.

Taussky HH, Shorr E. (1953). A microcolorimetric method for the determination of inorganic phosphorus. J Biol Chem. 202: 675-685.

Taylor RD, Jewsbury PJ, Essex JW. (2002). A review of protein-small molecule docking methods. J Comput Aided Mol Des. 16: 151-166.

Thowfeik FS (2016) Targeting a common enemy: Toxic cellular mechanism of novel anti-cancer agents that alter DNA and transcription. University of Cincinnati.

Tomlin C (1997) The Pesticide Manual, British Crop Protection Council. Farnham, Surrey, UK.

Tomlin CDS. (2004). The e-Pesticide Manual Version 3.1. London, UK. The British Crop Protection Council.

Tope AM, Panemangalore M. (2007). Assessment of oxidative stress due to exposure to pesticides in plasma and urine of traditional limited-resource farm workers: formation of the DNA-adduct 8-hydroxy-2-deoxy-guanosine (8-OHdG). J Environ Sci Health Part B. 42: 151-155.

Uehara H, Itoigawa Y, Wada T, Morikawa D, Koga A, Nojiri H, Kawasaki T, Maruyama Y, Ishijima M. (2021). Relationship of superoxide dismutase to rotator cuff degeneration and tear in a rat model. J Orth Res. 1-10.

Umemura T, Kodama Y, Kurokawa Y, Williams GM. (2000). Lack of oxidative DNA damage or initiation of carcinogenesis in the kidneys of male F344 rats given subchronic exposure to p-dichlorobenzene (pDCB) at a carcinogenic dose. Arch Toxicol. 74: 54-59.

Vessey DA, Boyer TD. (1984). Differential activation and inhibition of different forms of rat liver glutathione S-transferase by the herbicides 2,4-dichlorophenoxyacetate (2,4-D) and 2,4,5-trichlorophenoxyacetate (2,4,5-T). Toxicol Appl Pharmacol. 73: 492-499.

Villaverde JJ, B. S-M, C. Lp-G, Alonso-Prados J, Sandín-España P. (2017). Computational methodologies for the risk assessment of pesticides in the European Union. J Agric Food Chem. 65: 2017-2018.

Wang X, Wang W, Li L, Perry G, Lee H, Zhu X. (2014). Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochim Biophys Acta-Mol Bas Dis. 1842: 1240-1247.

Wei X, Zhu X, Jiang L, Long M, Du Y. (2021). Recent research progress on the role of ulinastatin in chronic kidney disease. Nephrology. 26: 708-714.

Zafiropoulos A, Tsarouhas K, Tsitsimpikou C, Fragkiadaki P, Germanakis I, Tsardi M, Maravgakis G, Goutzourelas N, Vasilaki F, Kouretas D, Hayes A, Tsatsakis A. (2014). Cardiotoxicity in rabbits after a low-level exposure to diazinon, propoxur, and chlorpyrifos. Hum Exp Toxicol. 33: 1241-1252.