Egypt. J. Chem. **Vol. 68**, No. 1 pp. 253– 267 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Genotoxicity of profenofos on weanling male rats: antioxidant and protective activity of artichoke leaves extract

Abdel-Tawab H. Mossa, Marwa F. Gad*, Samia M.M. Mohafrash

Pesticide Chemistry Department, Chemical Industries Research Institute, National Research Centre, Giza, Egypt, 33 El Bohouth Street (former El Tahrir St.), P.O. 12622, Dokki, Giza, Egypt.

Abstract

Organophosphorus insecticides (OPIs) are a common type of pesticides that have been mostly studied in adult animals, but their effects on early life stages are not well understood. In this study, we aimed to evaluate the genotoxic effect of profenofos (PFF), an OPI, on weanling male rats. The protective effect of Artichoke leaves extract (ALE), a natural antioxidant, against PFF-induced genotoxicity was investigated. ALE contains high amounts of phenolic and flavonoid compounds, such as gallic acid and chlorogenic acid, as determined by HPLC analysis. The extract also showed strong antioxidant activity in vitro, as measured by reducing power, DPPH●, and ABTS●+ assays. Weanling male rats was exposed to PFF at a dose of 14.32 mg/kg b.wt (1/25 LD50) with or without ALE at doses of 100 mg/kg and 200 mg/kg in drinking water for 28 days. We assessed the genotoxic and cytotoxic effects of PFF on the bone marrow cells using chromosomal aberrations, micronucleus, and mitotic index assays. The results showed that PFF significantly increased the frequency of chromosomal aberrations and micronucleus formation in the bone marrow cells and decreased the mitotic index in the exposed rats. The ALE reduced the genotoxic and cytotoxic effects of PFF on the bone marrow cells by decreasing the chromosomal aberrations and micronucleus formation and increasing the mitotic index. These findings suggest that PFF is a potential clastogenic/genotoxic agent, especially on early life stages that are more susceptible to its toxicity. The ALE can be used as a natural antioxidant and a protective agent against PFF-induced genotoxicity.

***Keywords*:** Antioxidant, Free radical, Artichokes, genotoxicity, micronucleus, chromosomal aberration, PFF, weanling rats

1. Introduction

In actuality, as people are constantly interacting with their surroundings, they are exposed to a wide range of synthetic chemicals that are present in their food, water, and environment. In both industrialized and developing nations, a broad variety of synthetic pesticides have been dispersed into rural areas through agricultural operations to control agricultural pests, insect pests, plant infections, and weeds [1]. In our previous work, commercial formulations of chlorpyrifos, cypermethrin, deltamethrin, fipronil, prallethrin, cyromazine and methomyl had higher adverse effects on biochemical and haematological markers in male rats [2], [3], [4], [5], [6]. Nevertheless, in addition to the active ingredient(s), pesticide formulations also contain a variety of other components, including a solvent, wetting and emulsifying agents, and additives [3], [4], [5], [7]. As a result, it is uncommon to be aware of the potential cumulative toxic consequences of such complex exposures. In order to assess the potential of adverse health consequences from pesticide exposure, toxicological information about active chemicals or formulations alone is not adequate [1], [7], [8], [9].

Pesticides commonly have high reactivity and are capable of forming covalent connections with a variety of biological macromolecules' nucleophilic sites, including DNA [10], [11], [12]. The indiscriminate use of pesticides may have negative consequences on human health due to their biological activity. For instance, the production of DNA damage has the potential to cause cancer, poor reproductive results, and a variety of other chronic illnesses [13], [14], [15], [16], [17]. According to epidemiological studies, occupational exposure to various pesticides may increase the risk of leukemia and other cancers [13], [17], [18], [19], [20].

The genotoxic effect of pesticides in vitro and in vivo has been documented by several researchers. In rat bone marrow cells, chromosomal aberration (CA), micronucleus (MN), and mitotic index (MI) were evaluated [21], [22], [23], [24]. Many pesticides, including chlorpyrifos, cypermethrin, methomyl, deltamethrin, and prallethrin, elevated CAs and MNs while lowering MI in the rat bone marrow cells were reported in our experiments [25], [26], [27], [28]. The MN test was performed to evaluate the genotoxicity of nuvacron (Monocrotophos) on Chinese hamster ovary (CHO) cells both in vitro and in in vivo. Mice's bone marrow had more polychromatic erythrocytes with a micronucleus after receiving doses of nuvacron of 2.5 and

*Corresponding author e-mail: marwagad885@gmail.com (Marwa f. Gad).
Receive Date: 21 March 2024,Revise Date: 14 May 2024,Accept Date: 20 May 2024
DOI: 10.21608/EJCHEM.2024.278550.9492

5.0 mg/kg intraperitoneally for 24 hours [29]. Additional pesticides including pirimiphos-methyl, dimethoate, lindane, endosulfan, chlorpyrifos, and monocrotophos have also been documented to cause CAs and MNs [30], [31], [32].

The mechanism by which pesticides cause mutagenesis is complex and reactive oxygen species play a significant role in pesticide toxicity, mutagens and carcinogens [33], [34], [35]. Other studies reported that correlation between ROS, mutation, and carcinogenesis [36]. ROS may play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation, which may be related to cancer, heart disease, and aging [37].

Natural extracts have been attracting a lot of attention recently because of their antioxidant, antimutagenic, and protective properties in both in vivo and in vitro studies [38].This is because chemical compounds that are used as drugs, food additives, or dietary supplements might have adverse effects on human health.For their possible use as drugs, food additives, dietary supplements, and antioxidants, natural compounds therefore serve as a respectable substitute for manufactured [38], [39], [40], [41].

Globe artichoke is a valuable crop that offers many health benefits due to its high content of nutrients and phenolic compounds [42]. These compounds have antioxidant, anti-inflammatory, and anticancer properties, among others. However, the majority of the globe artichoke plant is wasted after harvest, as only the immature flower heads are consumed. The leaves, stems, and roots, which account for nearly 85% of the plant's biomass, are usually discarded or used as animal feed. This represents a significant loss of potential resources and income for farmers and consumers [42], [43], [44]. Therefore, there is a need to explore alternative uses and applications for the globe artichoke by-products, such as extracting bioactive compounds, producing biofuels, or developing new food products. Therefore, one goal of this study was to extract the bioactive compounds from the leaves of Artichoke plant using aqueous ethanol as a solvent. However, antioxidant compounds have significant practical and therapeutic benefits. One of the main sources of these compounds are phenolic and flavonoids substances, which exhibit antioxidant, antimutagenic, anticancer and other biological effects [2], [26], [45], [46]. Plants that contain high amounts of these substances can be used as natural antimutagens in food and medicine to prevent mutations that may lead to cancer or other diseases caused by genotoxic agents [40], [47], [48], [49].

ALE and its protective effects were investigated in several studies using human and animal models. ALE reduced oxidative stress in human leukocytes, as measured by flow cytometry and dichlorofluorescin diacetate [38], [50]. It has also been shown to protect the liver from damage caused by free radicals, low-density lipoprotein oxidation, lipid peroxidation, and protein oxidation, as well as to enhance the activity of glutathione peroxidase, an enzyme that detoxifies hydrogen peroxide. These findings suggest that ALE has beneficial effects on oxidative stress and organ function in various settings [51], [52], [53].

Most studies on OPIs toxicity have only examined adult animals, leaving a gap in the knowledge of the consequences of exposure during early life stages. Previous research suggest that organophosphates and other pesticides can impair the biodiversity and health of living organisms, by causing cellular damage, mutations, genome degradation, population changes, diseases and cancer [24], [30], [54], [55]. Therefore, this study is the first of its kind to investigate the genotoxic effect of PFF, an organophosphorus pesticide commonly used in agriculture, on weanling male rats. We measured the phenolic and flavonoid content and the *in vitro* antioxidant activity of artichoke leaf extract. We also assessed the protective role of ALE against the genotoxicity induced by PFF in weanling male rats.

2. Materials and methods

2.1. Insecticides

Profenofos (Deliron El-Nasr 72% EC) was obtained from National Agricultural Chemicals and Investment, Egypt.

2.2. Chemicals and reagents

Chemicals and reagents such as ethanol, methanol, glycerol, Giemsa, Fetal Calf Serum (FCS) and potassium chloride were obtained from the local scientific distributors in Egypt. Giemsa were obtained from S.d. fine-chem. Ltd, Colchicine from BDH, England; May-Grónwald's from S.D. Fine Chem Limited, Mumbai, India; Fetal Calf Serum (FCS) from BioSource International, USA and potassium chloride from HMRZEL laboratories Ltd., Netherlands. All other chemicals were of reagent grades and obtained from the local scientific distributors in Egypt.

2.3. Plant materials and extraction

The artichoke leaves (*Cynara scolymus* L.) were collected from the Experimental Farm of the Faculty of Agriculture, Cairo University, Giza, Egypt, in 2021. The leaves were rinsed with tap water and then dried. The extraction process involved powdered leaves that were macerated with 70% aqueous ethanol.

2.4. Total phenolic content

The Folin-Ciocalteu method was used to measure the total phenolic content of artichoke extract. The procedure was as follows: A diluted solution of the extract (100 µL) was mixed with 500 µL of Folin-Ciocalteu reagent (0.2 N) and left for 5 min at room temperature. Then, 400 µL of sodium carbonate solution (75 g/L in water) was added to the mixture and the absorbance was measured at 765 nm after 1h, using water as a blank [56], [57]. A standard curve was constructed with gallic acid (0-300 mg/L) and the total phenolic content was calculated as mg of gallic acid equivalents (GAE) per g of dry weight

(DW) of the extract.

2.5. Total flavonoid content

The flavonoid content of artichoke extract was quantified by a spectrophotometric method. A solution of artichoke extract (0.5 ml) was mixed with 0.5 ml of 2% methanolic aluminum trichloride ($AlCl_3$) and left to stand for 15 minutes [56], [57]. The absorbance of the resulting complex was measured at 415 nm against a blank consisting of 0.5 ml of artichoke extract and 0.5 ml of methanol without $AlCl_3$. The flavonoid concentration was calculated from a standard curve of quercetin and expressed as mg of quercetin equivalents (QE)/g of dry weight (DW).

2.6. In vitro antioxidant activity

2.6.1. DPPH• (2,2´-diphenylpicrylhydrazyl) scavenging activity

The aqueous ethanol extract of artichokes was tested for its ability to scavenge DPPH• radicals, using ascorbic acid as a standard. The procedure involved mixing 1.0 ml of the extract at various concentrations (5-100 µg/ml) with 1.0 ml of 0.1 mM DPPH• in methanol, and then adding MeOH to make the final volume 3.0 ml. The mixture was shaken well and left in the dark at room temperature for 30 min, after which the absorbance was read at 517 nm [56], [57]. Methanol was used as a blank and ascorbic acid (0.5-10 µg/ml) was used as a reference compound. The absorbance of solvent and DPPH• radical without extract was taken as a control. The percentage inhibition of DPPH• by the samples was calculated using the following formula:

$$I\ (\%) = [(A_C - A_S)/A_C] \times 100$$

Where: A_C and A_S are the absorbance of the control and sample, respectively. The IC_{50} value represents the concentration of the extract, which caused 50% inhibition was calculated.

2.6.2. ABTS•+ (2, 2'azinobis- (3-ethylbenzthiazoline -6-sulphonic acid)) scavenging activity

The antioxidant activity of aqueous ethanol extract of artichokes and ascorbic acid was studied using $ABTS^{\bullet+}$ radical scavenging activity. The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Because ABTS and potassium persulfate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the ABTS [56], [57] . Oxidation of the ABTS commenced immediately, but the absorbance was not maximal. The radical was stable in this form for more than two days when stored in the dark at room temperature. Before use, the $ABTS^{\bullet+}$ solution was diluted with phosphate buffer (0.1 M, pH 7.4) to get an absorbance of 0.70 ± 0.02 at 734 nm. Then, 1 ml of $ABTS^{\bullet+}$ solution was added to 1 ml of extract solution in distilled water at different concentrations (5-80 µg/ml). After 30 min, the percentage inhibition at 734 nm was calculated for each concentration relative to a blank absorbance. Ascorbic acid (0.5-10 µg/ml) was used as the reference compound. The scavenging capability of $ABTS^{\bullet+}$ radical was calculated using the following equation:

$$ABTS^{\bullet+}\ \text{Scavenging effect}\ (\%) = [(A_{Control} - A_{Sample})/A_{Control})] \times 100$$

Where $A_{Control}$ is the absorbance of the control and A_{Sample} is the absorbance of the sample. The IC_{50} value represents the concentration of the extract, which caused 50% inhibition was calculated

2.6.3. Reducing power

To measure the total reducing power of the artichoke's aqueous ethanol extract and ascorbic acid was applied. A mixture of one ml of extract (5-60 µg/ml), 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% $K_3\ Fe\ (CN)_6$ was prepared and incubated at 50°C for 20 minutes. Then, 2.5 ml of 10% TCA was added and the mixture was centrifuged [56], [57]. The absorbance of the supernatant (2.5 ml) mixed with 2.5 ml of water and 0.5 ml of 0.1% $FeCl_3$ was read at 700 nm for concentrations ranging from 5 to 15 µg/ml.

2.7. HPLC analysis

An Agilent 1260 series high-performance liquid chromatography (HPLC) system was used to perform the chromatographic analysis. The Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm) was employed for the separation of the analyses. The mobile phase was composed of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) and delivered at a flow rate of 1 ml/min. A linear gradient elution was applied as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A) and 15-16 min (82% A). The detection wavelength was set at 280 nm using a multi-wavelength detector. The sample solutions were injected with a volume of 10 µL each. The temperature of the column oven was controlled at 35 °C.

In this study, sixteen phenolic compounds as standards, covering different classes and subclasses of phenolics was used. These compounds are Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Coffeic acid, Syringic acid, Pyro catechol, Rutin, Ellagic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, Taxifolin, Cinnamic acid and Kaempferol.

2.8. In vitro studies

2.8.1. Animals and treatments

The experiment involved weanling male Wistar rats (average body weight of 55±5 g) that were procured from the Animal Breeding House of the National Research Centre (NRC), Dokki, Cairo, Egypt. The rats were kept in clean plastic cages and

had free access to food (standard pellet diet) and tap water ad-libitum. The rats were maintained under standardized housing conditions (12 h light/dark cycles, the temperature was 22±4 ºC and a minimum relative humidity of 46%) in the laboratory animal room. The animals were treated humanely, following the guidelines in the "Guide for the Care and Use of Laboratory Animals"[58].

2.8.2. Experimental design

The experimental design was as follows: Five groups of rats (n=5 per group) were acclimatized to the laboratory environment for a week before the treatment. Group 1 was the control group and received corn oil (1ml/kg b.wt) daily by oral administration. Group 2 was exposed to PFF at a dose of 14.32 mg/kg b.wt (1/25 LD_{50}), based on the reported LD_{50} value of 358 mg/kg b.wt [59]. Groups 3 and 4 received the same dose of PFF as group 2, along with Artichokes extract, a natural antioxidant, at doses of 100 mg/kg and 200 mg/kg in water, respectively. The dose of 200 mg/kg was chosen as the highest safe dose, based on a previous study that showed no toxicity at 2000 mg/kg ($1/10^{th}$ of this dose was used), and the lower dose was half of the higher dose. Group 5 received only Artichokes extract at 200 mg/kg daily. The treatment lasted for 28 days and the doses were adjusted weekly according to the body weight changes of the rats.

2.8.3. Chromosome aberrations (CA) assay

The direct method of rinsing marrow of long bones, as described by Adler [60], to perform cytogenetic analysis was used. We injected colchicine (4 mg/kg) intraperitoneally to experimental animals 1.5h before euthanasia. We removed and cleaned both femurs from any muscle tissue. We flushed bone marrow cells from both femurs in KCl (0.075M, at 37ºC) and incubated them at 37ºC for 25min. We spun the cells at 2000 rpm for 10 min using Hereaeus Labofuge 400R, Kendro Laboratory Products GmbH, Germany, and fixed them in acetone-methanol (acetic acid: methanol, 1:3 v/v). We repeated centrifugation and fixation five times with a 20 min interval. We re-suspended the cells in a small volume of fixative, dropped them onto chilled slides, and let them dry. We stained them the next day with freshly prepared 2% Giemsa stain for 3-5 min, and washed them in distilled water to remove excess stain. We screened 100 metaphases per animal for treatments and control using OPTIKA Microscopes. We identified CA based on the OECD Guideline 475 criteria, updated and adopted on Adopted: 29 July 2016 [61]. We calculated the percentage reduction of chromosomal aberrations as follows:

Reduction (%) = 100 - [(percent of frequency of aberrant cell in PFF + Artichokes extract / percent of frequency of aberrant cell in PFF) x 100]

2.8.4. Mitotic index determination

To measure the mitotic index, we used the same slides that we prepared for the chromosomal aberration assay. We counted the number of cells in mitosis and the total number of cells for each animal in the control and treatment groups, using a Nikon microscope. We divided the number of mitotic cells by the total number of cells and multiplied by 100 to get the mitotic index for each animal.

2.8.5. The micronucleus assay

The protocol of Schmid [62] was used to measure the micronuclei (MN) frequency in polychromatic erythrocytes (PCEs) from rat bone marrow. We euthanized the rats, extracted the femurs and collected the bone marrow in 2 ml of Fetal Calf Serum. We centrifuged the cell suspension and prepared a thin smear of the cell pellet on the slides, which we air-dried and fixed in pure methanol. We made at least four slides per animal, dried them overnight and stained them with May-Grunwald/Giemsa [63]. We blinded the slides for microscopic examination. We counted 2000 PCEs per animal from four randomly chosen slides and recorded the MN occurrence at 1000× magnification with a light microscope. The study complied with OECD Guideline 474, revised and adopted on Adopted: 29 July 2016 [64].

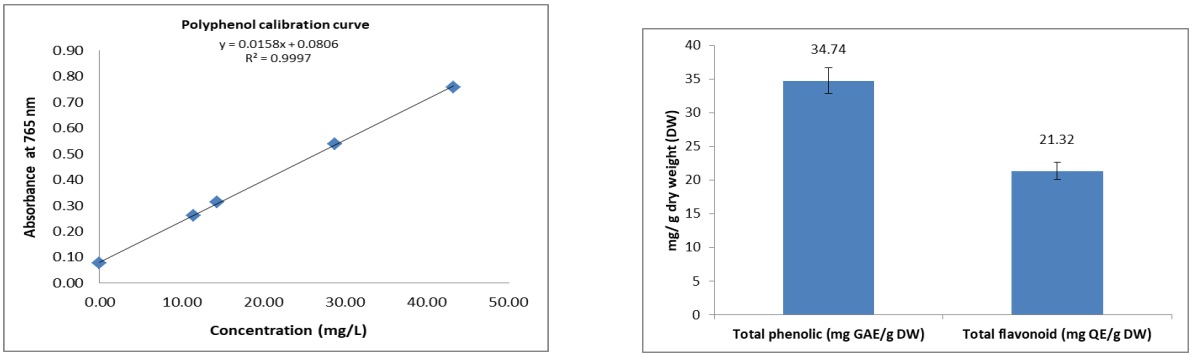
2.9. Statistical analysis

One-way ANOVA analysis with post hoc multiple comparisons were used to perform the statistical analysis of the data. The data analysis was done using SPSS version 18.0 for Windows (SPSS Inc. 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412), a software for statistical computing and graphics.

3. Results :

3.1. Total phenolic and flavonoid contents

As shown in Figure 1, the artichoke aqueous ethanol extract exhibited high levels of total phenolic (TPC) and flavonoid (TFC) compounds. The TPC and FC were measured using the polyphenol calibration curve and found to be 34.74 ± 1.88 mg GAE/g DW and 21.32 ± 1.28 mg QE/g DW, respectively, in the artichoke extract.



A B

Figure 1. Standard curve of gallic acid "polyphenol calibration curve" (A) and total phenolic (TPC) and flavonoid (TFC) contents in artichokes leaves extract (B). The value represents mean ± SE of five determinations.

3.2. DPPH• and ABTS•+ scavenging activity

The artichoke aqueous ethanol was tested for its ability to neutralize free radicals using DPPH and ABTS assays. The extract showed a dose-dependent increase in radical scavenging activity in both assays. Figure 2 illustrates that the artichoke extract had an IC_{50} of 45.32 µg/ml for DPPH and 27.31 µg/ml for ABTS, while the reference compound, ascorbic acid, had an IC_{50} of 6.48 µg/ml for DPPH and 4.67 µg/ml for ABTS, respectively. These results indicate that the ALE has a high potential as a natural antioxidant.

3.2.1. Reducing power

The test solution's yellow color shifts to different shades of green and blue depending on the extract's reducing power in the reducing power assay. Thus, a higher absorbance of the sample with concentrations implies a higher reducing potential of the samples. The reducing power of artichoke's aqueous ethanol extract was measured at concentrations ranging from 5 to 60 µg/ml (Figure 3) in this study. The reduction power increased as the concentration increased. This result suggested that ALE had a high reducing power. However, the reducing powers of ascorbic acid only increased from 5- 15 µg/ml as the concentration increased.

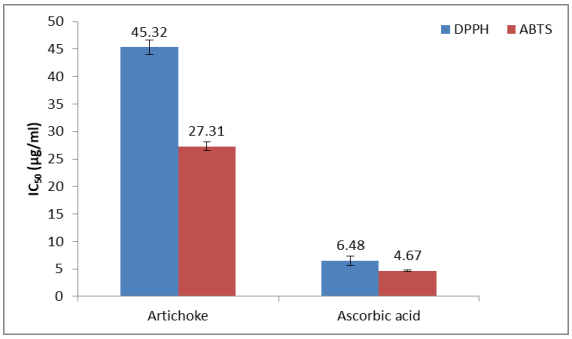


Figure 2. DPPH and ABTS radical scavenging activity of aqueous ethanolic extract of artichoke. The value represents mean ± SE of five determinations. IC_{50} values represent the concentration of the extract, which caused 50% inhibition in DPPH• or $ABTS^{•+}$.

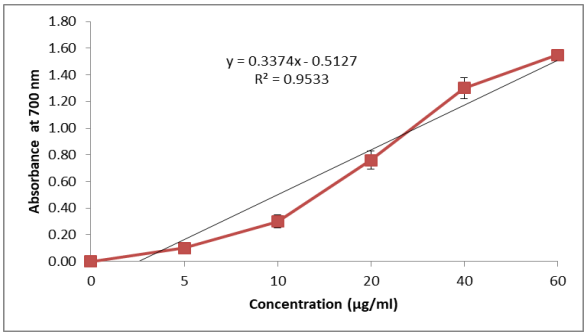


Figure 3. Reducing power of aqueous ethanolic extract of artichokes. The value represents mean ± SD of five determinations.

3.2.2. HPLC analysis

The phenolic profile of artichoke extract using sixteen phenolic standards from different classes and subclasses of phenolic compounds were analyzed. Table 1 and Figure 4 show the concentrations (µg/g) of various phenolic compounds in artichoke extract. Gallic acid (4072.48 µg/g) and chlorogenic acid (3348.80 µg/g) were the most abundant compounds, while catechin, pyro catechol, rutin, ferulic acid and kaempferol were not detected. Other phenolic compounds in artichoke extract included methyl gallate (52.98 µg/g), coffeic acid (55.89 µg/g), syringic acid (163.81 µg/g), fllagic acid (135.67 µg/g), coumaric acid (4.49 µg/g), vanillin (534.85 µg/g), naringenin (1552.15 µg/g) and taxifolin (31.56 µg/g).

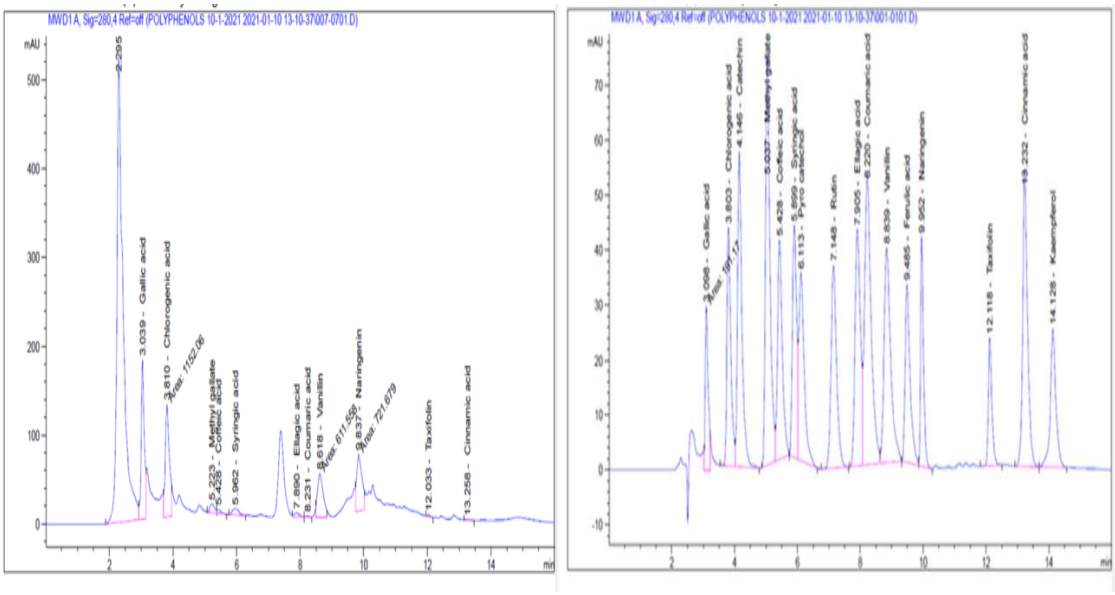


Figure 4. HPLC analysis of standard phenolic acid and the aqueous ethanolic extract of artichokes.

Table 1. HPLC analysis of phenolic compound in aqueous ethanolic extract of artichokes.

Phenolic compound	Area	Conc.(µg/g extract)
Gallic acid	1190.97	4072.48
Chlorogenic acid	1152.06	3348.80
Catechin	0.00	0.00
Methyl gallate	100.30	52.98
Coffeic acid	33.61	55.89
Syringic acid	96.20	163.81
Pyro catechol	0.00	0.00
Rutin	0.00	0.00
Ellagic acid	52.11	135.67
Coumaric acid	6.79	4.49
Vanillin	611.56	534.85
Ferulic acid	0.00	0.00
Naringenin	721.68	1552.15
Taxifolin	11.16	31.56
Cinnamic acid	6.72	2.64
Kaempferol	0.00	0.00

3.3. In vivo studies

3.3.1. Mitotic index

The rate of cell division was measured by the mitotic index, which is the percentage of cells undergoing mitosis. The control group of rats had a mitotic index of 8.28 ±0.32, and the group treated with Artichoke extract had a similar value of 8.31±0.84. However, the group exposed to PFF, a pesticide, showed a significant decrease in the mitotic index (5.33 ±0.24 P< 0.05), indicating that PFF has a cytotoxic effect on the cells. The group that received both PFF and Artichoke extract had a higher mitotic index than the PFF-only group, suggesting that Artichoke extract can protect the cells from PFF-induced cytotoxicity. The protective effect was more pronounced at the higher dose of Artichoke extract (200 mg/kg) than at the lower dose (100 mg/kg). The mitotic index of this group was 7.89 ±0.46, which was not significantly different from the control group (Figure

5). This result demonstrates the anti-cytotoxic potential of Artichoke extract against PFF exposure.

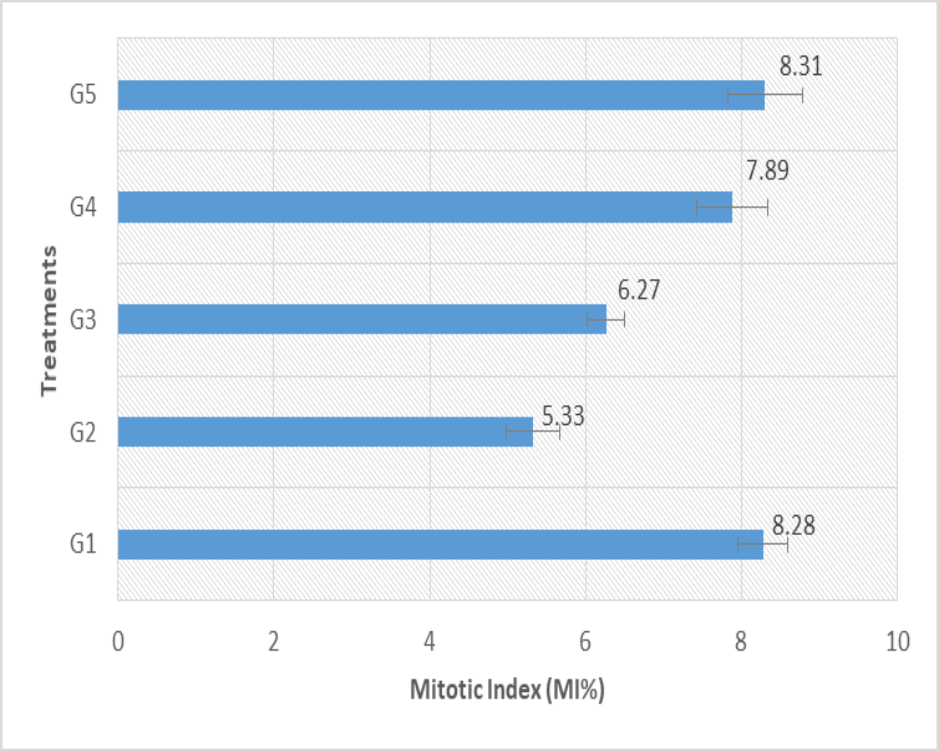


Figure 5. Mitotic Index (MI %) of treated rats and the protective effect of Artichokes extract. Values are mean ± SE, Control (G1), profenofos (G2), profenofos + artichokes at 100 mg/kg (G3), profenofos + artichokes at 200 mg/kg (G4) and artichokes at 200 mg/kg (G5).

3.3.2. Chromosomal aberrations in rat bone marrow

The effects of PFF and Artichokes extract on the chromosomal integrity of rat bone marrow cells are shown in Table 2. It was observed that PFF at a dose of 14.32 mg/kg. b.wt. caused a significant increase in the frequency of chromosomal aberrations (CA) in bone marrow cells, excluding gaps, compared to the control group (1.50 ±0.002 vs 11.06±0.013). Artichokes extract alone did not induce any significant CA (1.45±0.007). However, when Artichokes extract was administered to PFF-treated rats, it reduced the mutagenic damage caused by PFF. The percentage of CA, excluding gaps, decreased by 80.01% and 27.49% when rats were treated with Artichokes extract at doses of 200 mg/kg. b.wt and 100 mg/kg. b.wt, respectively, along with PFF. The types of CA induced by PFF, such as gaps, breaks, fragments, deletions and multiple aberrations, were also reduced by Artichokes extract treatment, especially at the high dose (200 mg/kg. b.wt). The number of aberrations per cell also decreased by Artichokes extracts administration (Table 2).

3.3.3. Induction of micronuclei in rat bone marrow PCE

Data in Table 3 show that the tested insecticide PFF induced a potential clastogenic effect in the bone marrow of treated rats as evidenced by the significant increase (23.24±1.32) in the total number of bone-marrow micronucleated polychromatic erythrocytes (MnPCE), when compared with control rats (5.95± 0.11). The simultaneous administration of *Artichokes* at 100 mg/kg. b. wt. with PFF significantly decreased (11.68±0.44) and decreased to 7.09±0.43after administration *Artichokes* at 200 mg/kg. b. wt. respectively. *Artichokes* reduce micronucleated polychromatic erythrocytes (MnPCE) in rat bone marrow by 49.74% and 69.49 at doses 100 and 200 mg/kg. b.wt, respectively. The obtained data revealed that the treatment with PFF at tested dose caused a significant increase in the frequencies of micronucleated polychromatic erythrocytes (PCEM).

Table 2. Effect of profenofos on chromosomal aberrations in rat bone marrow cells and the protective role of Artichokes extract.

Group	Percent chromosome aberrations (Mean ± S.E.)				Total aberrant cell (%) (Mean ± S.E.)				No. of aberrations per cell (Mean ± S.E.)	
	Gaps	Breaks and/ or Fragment	Deletions	Multiple aberrations	Including gaps	Reduction (%)	Excluding gaps	Reduction (%)	Including gaps	Excluding gaps
G1	**1.14±0.05**	**1.18±0.1**	**0.17±0.002**	**0.15±0.002**	**2.64±0.039^{d}**	**-**	**1.50±0.002^{c}**	**-**	**0.026±0.001**	**0.015±0.0001**
G2	**3.45±0.08**	**4.99±0.09**	**0.86±0.003**	**5.21±0.006**	**14.51±0.045^{a}**	**-**	**11.06±0.013^{a}**	**-**	**0.145±0.002**	**0.111±0.001**
G3	**2.25±0.06**	**3.84±0.07**	**0.54±0.004**	**3.64±0.003**	**10.27±0.043^{b}**	**29.22**	**8.02±0.05^{b}**	**27.49**	**0.103±0.001**	**0.080±0.0003**
G4	**1.63±0.02**	**1.42±0.03**	**0.23±0.004**	**0.34±0.02**	**3.62±0.019^{c}**	**75.05**	**1.99±0.006^{c}**	**80.01**	**0.036±0.001**	**0.020±0.0001**
G5	**1.11±0.001**	**1.13±0.02**	**0.18±0.001**	**0.14±0.001**	**2.56±0.011^{d}**	**-**	**1.45±0.007^{c}**	**-**	**0.026±0.001**	**0.015±0.0001**

Value is mean ± S.E.; n = 5 rats/group. Values are shared the same superscript letters not differ significantly at $p < 0.05$. Values are mean ± SE, Control (G1), profenofos (G2), profenofos + artichokes at 100 mg/kg (G3), profenofos + artichokes at 200 mg/kg (G4) and artichokes at 200 mg/kg (G5).

Reduction (%) = 100 - [(percent of frequency of aberrant cell in profenofos + Artichokes extract / percent of frequency of aberrant cell in profenofos) x 100]

Table 3. Effect of profenofos on micronuclei in rat bone marrow cells and the protective role of *Artichokes* extract.

Group	No. of micronucleated polychromatic erythrocytes (MnPCE) (Mean ± S.E.)				Reduction (%)
	PCE with one Mn	PCE with two Mn	PCE with more than two Mn	Total MnPCE	
G1	5.30±0.035	0.50±0.001	0.15±0.001	5.95± 0.11^{d}	-
G2	14.4±0.76	6.49±0.21	2.35±0.08	23.24±1.32^{a}	-
G3	7.43±0.98	2.85±0.011	1.40±0.05	11.68±0.44^{b}	49.74
G4	5.55±0.26	1.22±0.11	0.32±0.01	7.09±0.43^{c}	69.49
G5	5.28±0.45	0.46±0.001	0.14±0.001	5.88±0.31^{d}	

Value is mean ± S.E.; n = 5 rats/group. The number of the scored cells was 2000 cells/ animal. Values are shared the same superscripts letters not differ significantly at $p < 0.05$. Control (G1), profenofos (G2), profenofos + *Artichokes* at 100 mg/kg (G3), profenofos + *Artichokes* at 200 mg/kg (G4) and *Artichokes* at 100 mg/kg (G5). Mn: micronucleus, MnPCE: micronucleated polychromatic erythrocytes, PCE: polychromatic erythrocytes.

Reduction (%) = 100 - [(total MnPCE in profenofos + *Artichokes* extract /total MnPCE in profenofos) x 100]

4. Discussion

Synthetic pesticides are applied to crops and public health settings to control or manage pests. They are considered effective tools for increasing agricultural productivity and preventing diseases transmitted by insects and rodents [65]. However, they also pose risks to human health, animal welfare and ecosystem. One of the mechanisms by which pesticides cause damage is by inducing oxidative stress in various biological systems. Oxidative stress is a condition where the levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) exceed the capacity of the antioxidant defense system, leading to cellular damage and dysfunction [3], [4], [5], [6], [12], [27], [28]. Oxidative stress has been implicated in many chronic diseases, such as cancer, inflammation, cardiovascular and neurodegenerative disorders. ROS and RNS can also trigger apoptotic pathways that result in programmed cell death [66], [67]. Some studies have suggested that antioxidants from natural or synthetic sources can counteract or mitigate the oxidative stress induced by pesticides.

The artichoke plant is a source of secondary metabolites of the phenolic type, such as flavonoids and phenolic acids [38], [43], [51]. These metabolites have antioxidant activity and can be applied in traditional medicine to prevent or treat various disorders. We quantified the total phenolic (TPC) and flavonoid (FC) content of an aqueous ethanol extract of artichoke and obtained 34.74 ± 1.88 mg GAE/g DW and 21.32 ± 1.28 mg QE/g DW, respectively. These results suggest a high presence of

phenolic compounds in the extract. We evaluate the antioxidant potential of artichoke extract using established *in vitro* methods, such as reducing power, DPPH$^{\bullet}$, and ABTS$^{\bullet+}$, assays. The results indicated that the radical scavenging activity of the extract increased proportionally to the concentration in both assays. The IC_{50} values of the extract were 45.32 µg/ml for DPPH and 27.31 µg/ml for ABTS, while the IC_{50} values of the reference compound, ascorbic acid, were 6.48 µg/ml for DPPH and 4.67 µg/ml for ABTS, respectively. DPPH$^{\bullet}$ is a stable free radical with a high absorbance at 517 nm, which decreases when it reacts with an antioxidant to form 1,1-diphenyl-2-picrylhydrazine [68]. The absorbance change reflects the scavenging capacity of the antioxidant extract. The ABTS•+ assay is a powerful technique for measuring the antioxidant activity of substances that can donate hydrogen atoms (quenching radicals in the aqueous phase) and of substances that can interrupt the chain reaction of lipid oxidation (quenching lipid peroxyl radicals) [69]. The assay relies on the generation of a stable radical cation (ABTS$^{\bullet+}$) that can be reduced by antioxidants. The reduction of the radical cation is monitored by the decrease in its absorbance at 734 nm. In this study, we evaluated the reduction power of artichoke's aqueous ethanol extract at different concentrations from 5 to 60 µg/ml. We observed that the reduction power increased proportionally to the concentration.

The phenolic profile of artichoke extract was analyzed by high-performance liquid chromatography (HPLC) to identify and quantify the main compounds present in the sample. The results showed that gallic acid and chlorogenic acid were the most abundant phenolic compounds, with concentrations of 4072.48 and 3348.80 µg/g, respectively. Other phenolic compounds that were detected in the artichoke extract included methyl gallate (52.98 µg/g), coffeic acid (55.89 µg/g), syringic acid (163.81 µg/g), fllagic acid (135.67 µg/g), coumaric acid (4.49 µg/g), vanillin (534.85 µg/g), naringenin (1552.15 µg/g) and taxifolin (31.56 µg/g). The high content of phenolic and flavonoid compounds in artichoke extract may explain its scavenging and high antioxidant activity observed in the present study [70], [71], [72]. Gallic acid and chlorogenic acid, which are abundant in the extract, have been shown to exhibit high antioxidant activity. For example, gallic acid, or 3,4,5-trihydroxybenzoic acid, is a common trihydroxybenzoic acid that can be found in various plants [73]. It has a benzene ring with three hydroxyl groups and one carboxylic acid group attached to it. The hydroxyl groups are in an ortho position, which means they are adjacent to each other on the benzene ring. This gives gallic acid a flat and bent shape, which enhances its ability to act as an antioxidant [73], [74]. Chlorogenic acids (CGAs) are a group of plant polyphenols with a distinctive chemical structure. They are formed by the esterification of quinic acid with cinnamic acids, such as caffeic, ferulic, and p-coumaric acids. These cinnamic acids have hydroxyl groups on adjacent carbon atoms of the aromatic ring [75], [76].

In addition, other phenolic acids are widely distributed in plants and have various antioxidant and biological activities.

Protecting children from xenobiotics, which are substances that are foreign to the body, is a major concern for researchers and social workers. Children have higher vulnerability to these substances, which can come from various sources of pollution [77], [78]. Children's exposure to xenobiotics can affect their health and development in many ways, such as causing brain disorders, cancer, hormone problems, and kidney issues. These effects may not be apparent until later in life, so children have longer latency periods than adults. Therefore, it is important to develop better methods to assess and reduce the risks that children face from xenobiotics at different stages of their life cycle, from prenatal to youth [78], [79], [80]. In addition, children's bodies and organs are still growing and developing, and they can be more affected by pollutants from different sources. They may also get more exposure than adults because of their size and behavior may. Furthermore, early exposure to these exposures can interfere with the normal development and growth of children in important periods of their lives [81]. Children also have a longer lifespan than adults, which means they have more chances to develop diseases with long periods of time between exposure and symptoms, such as neurological disorders, cancer, endocrine disorders, and kidney problems [82], [83], [84].

Pesticides are also have harmful effects on human health, especially for children who are more vulnerable to their toxicity. A number of scientific studies have found associations between pesticide exposure and various types of childhood cancer, such as leukemia, lymphoma, brain tumors, and neuroblastoma [85], [86], [87]. These cancers affect the blood, the immune system, the nervous system, and other organs of the body. Childhood cancer is a serious public health problem that has been increasing in prevalence over the past decades. Pesticides may be one of the factors that contribute to this trend [88], [89], [90]. However, the majority of research on the toxic effects of organophosphates has been conducted on adult animals, leaving a gap in the knowledge of the consequences of exposure during lactation and weaning periods. These critical developmental stages may be more vulnerable to organophosphate-induced neurotoxicity and behavioral impairments [91], [92], [93].

One of the essential processes of cellular growth is cellular division. In this process, cells duplicate their DNA in S phase and then enter mitosis, which results in the formation of two daughter cells under normal circumstances. Furthermore, the level of mitotic activity reflects the level of cellular growth activity. The most basic and traditional way to measure mitotic activity (mitotic index (MI)) is to manually count the number of cells undergoing mitosis in a specific cell population of interest [94], [95]. The effect of PFF on cell division in weanling rats was assessed by measuring the mitotic index, or the percentage of cells in mitosis. The results showed that PFF-treated rats had significantly lower mitotic indices than control rats, suggesting that PFF caused cell damage. However, when the rats were co-administered with Artichoke extract, their mitotic indices increased, indicating that Artichoke extract could protect the cells from PFF-induced damage. The protective effect was more pronounced at the higher dose of Artichoke extract (200 mg/kg) than at the lower dose (100 mg/kg). The process of cell division and the mitotic index studies are important indicators of the health and viability of cells. However, some pesticides,

such as dimethoate, determination, and prallethrin, can have toxic effects on these processes and cause cellular damage. Several studies have documented the adverse effects of these pesticides on the cell cycle [22], [26], [96].

Chromosome aberrations refer to changes in the number or structure of chromosomes. These changes can impair the normal development and function of cells, tissues, and organs. Chromosome number aberrations involve having extra or missing chromosomes, which can lead to spontaneous abortions or serious congenital anomalies in newborns. Chromosome structure aberrations involve deletions, inversions, or exchanges of chromosome segments, which can result in birth defects, cancer, or other diseases that manifest later in life [97], [98], [99], [100]. Our results indicated that PFF had an impact on the chromosomal integrity of weanling rat bone marrow cells. PFF caused a significant increase in the frequency of chromosomal aberrations (CA) in bone marrow cells, excluding gaps, relative to the control group. Artichokes extract, on the other hand, mitigated the mutagenic damage induced by PFF in PFF-treated rats. The types of CA caused by PFF, such as gaps, breaks, fragments, deletions and multiple aberrations, were also reduced by Artichokes extract treatment, especially at the high dose (200 mg/kg. b.wt). The number of aberrations per cell also decreased by Artichokes extracts administration. Other studies have found significant differences in the frequency of CAs between individuals who were exposed to pesticides and those who were not [15], [101], [102].

The *in vivo* mammalian erythrocyte micronucleus test is a cytogenetic assay that can detect the potential of a test item to cause chromosomal damage and/or damage to the mitotic apparatus. This test is relatively simple and fast, and it involves the analysis of micronuclei in erythrocytes from peripheral blood or bone marrow of treated animals [103]. The results of this study indicate that PFF has a clastogenic effect on the bone marrow cells of weanling rats, as shown by the increased number of micronucleated polychromatic erythrocytes (MnPCE) in the treated rats compared to the controls. However, artichokes, a vegetable with antioxidant properties, can reduce the genotoxic damage caused by PFF, as evidenced by the decreased number of MnPCE in the bone marrow of weanling rats treated with artichokes and PFF. The data suggest that artichokes may have a protective effect against the chromosomal aberrations induced by PFF in rat bone marrow cells. However, micronucleus is a small fragment of chromosomal material that is separated from the main nucleus during cell division. Therefore, it is important to monitor the occurrence of micronucleus in exposed populations and to identify the pesticides that are responsible for this effect [104], [105], [106], [107], [108]. .

The mechanism of PFF induced cytotoxicity and genotoxicity could be due to the oxidative stress and DNA damage caused by its metabolites. PFF is an organophosphorus pesticide that inhibits acetylcholinesterase activity and disrupts the nervous system. It can also induce oxidative stress by generating reactive oxygen species (ROS) and depleting antioxidant enzymes. ROS can damage cellular macromolecules such as lipids, proteins and DNA, leading to cell death and mutagenesis. Moreover, cytochrome P450 enzymes to form more toxic compounds, such as PFF-oxon and PFF-sulfone, which can interact with DNA, can metabolize PFF and cause strand breaks, cross-links and adducts. These DNA lesions can impair the fidelity of DNA replication and transcription, resulting in genomic instability and carcinogenesis [109], [110], [111], [112], [113]. Artichoke may have a protective effect on cellular health by preventing or reducing the oxidative stress and genotoxicity caused by PFF. This is because artichoke contains phenolic compounds, which are antioxidants that can capture and neutralize ROS and free radicals. This can prevent or repair the oxidative damage and restore the cell function. These phenolic compounds can also act as electron donors to free radicals, making them less reactive and stopping the radical chain reaction [69], [114], [115], [116], [117].

5. Conclusion

The phenolic profile of ALE was determined by HPLC and revealed that gallic acid and chlorogenic acid were the predominant compounds, with concentrations of 4072.48 and 3348.80 µg/g, respectively. The antioxidant capacity of ALE was evaluated by different methods, such as reducing power, DPPH$^{\bullet}$, and ABTS$^{\bullet+}$, and showed high activity. The genotoxic and cytotoxic effects of PFF, an organophosphorus pesticide, were assessed in the bone marrow cells of weanling rats using chromosomal aberrations, micronucleus, and mitotic index assays. The results indicated that PFF induced significant chromosomal aberrations and micronucleus formation in the bone marrow cells and decreased the mitotic index in the exposed rats. The ALE exhibited anti-mutagenic and anti-cytotoxic properties and attenuated the damaging effects of PFF on the bone marrow cells. Therefore, PFF can be considered as a potential a clastogenic/genotoxic agent, especially for children who are more vulnerable to its toxicity. The present study suggests that ALE can be used as a natural source of antioxidants and as a protective agent against the genotoxicity and cytotoxicity of pesticides in agricultural and pesticide workers.

6. References

[1] M. A. Abbassy, A. E. S. M. Marei, M. A. M. Al-Ashkar, and A. T. H. Mossa, “Adverse biochemical effects of various pesticides on sprayers of cotton fields in El-Behira Governorate, Egypt,” *Biomed. Aging Pathol.*, vol. 4, no. 3, pp. 251–256, 2014, doi: 10.1016/j.biomag.2014.04.004.

[2] A. T. H. Mossa, A. A. Refaie, A. Ramadan, and J. Bouajila, “Amelioration of prallethrin-induced oxidative stress and hepatotoxicity in rat by the administration of origanum majorana essential Oil,” *Biomed Res. Int.*, vol. 2013, 2013, doi: 10.1155/2013/859085.

[3] S. A. Mansour and A. T. H. Mossa, “Oxidative damage, biochemical and histopathological alterations in rats exposed to

chlorpyrifos and the antioxidant role of zinc," *Pestic. Biochem. Physiol.*, vol. 96, no. 1, pp. 14–23, 2010, doi: 10.1016/j.pestbp.2009.08.008.

[4] S. A. Mansour and A. T. H. Mossa, "Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc," *Pestic. Biochem. Physiol.*, vol. 93, no. 1, pp. 34–39, Jan. 2009, doi: 10.1016/j.pestbp.2008.09.004.

[5] A. T. H. Mossa, E. S. Swelam, and S. M. M. Mohafrash, "Sub-chronic exposure to fipronil induced oxidative stress, biochemical and histotopathological changes in the liver and kidney of male albino rats," *Toxicol. Reports*, vol. 2, pp. 775–784, 2015, doi: 10.1016/j.toxrep.2015.02.009.

[6] T. M. Heikal, A. T. H. Mossa, M. A. Abdel Rasoul, and G. I. K. Marei, "The ameliorating effects of green tea extract against cyromazine and chlorpyrifos induced liver toxicity in male rats," *Asian J. Pharm. Clin. Res.*, vol. 6, no. 1, pp. 48–55, 2013.

[7] M. Abbassy and A. H. Mossa, "Haemato-biochemical effects of formulated and tech- nical cypermethrin and deltamethrin insecticides in male rats.," *J Pharmacol Toxicol*, vol. 7, pp. 312–321., 2012.

[8] H. N. Autrup, "Toxicity and assessment of chemical mixtures," 2012.

[9] F. R. Cassee, J. P. Groten, P. J. van Bladeren, and V. J. Feron, "Toxicological evaluation and risk assessment of chemical mixtures," *Crit. Rev. Toxicol.*, vol. 28, no. 1, pp. 73–101, 1998.

[10] R. O. Sule, L. Condon, and A. V. Gomes, "A Common Feature of Pesticides: Oxidative Stress - The Role of Oxidative Stress in Pesticide-Induced Toxicity," *Oxid. Med. Cell. Longev.*, vol. 2022, pp. 1–31, Jan. 2022, doi: 10.1155/2022/5563759.

[11] V. M. Pathak *et al.*, "Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review," *Front. Microbiol.*, p. 2833, 2022.

[12] A. A. Refaie, A. Ramadan, N. M. Sabry, W. K. B. Khalil, and A.-T. H. Mossa, "Synthetic insecticide fipronil induced over gene expression, DNA and liver damage in female rats: the protective role of fish oil," *Egypt. J. Chem.*, vol. 64, no. 5, pp. 2325–2336, 2021.

[13] R. Meinert, J. Schüz, U. Kaletsch, P. Kaatsch, and J. Michaelis, "Leukemia and non-Hodgkin's lymphoma in childhood and exposure to pesticides: results of a register-based case-control study in Germany," *Am. J. Epidemiol.*, vol. 151, no. 7, pp. 639–646, 2000.

[14] S. Mostafalou and M. Abdollahi, "Pesticides and human chronic diseases: evidences, mechanisms, and perspectives," *Toxicol. Appl. Pharmacol.*, vol. 268, no. 2, pp. 157–177, 2013.

[15] S. Mostafalou and M. Abdollahi, "Pesticides: an update of human exposure and toxicity," *Arch. Toxicol.*, vol. 91, no. 2, pp. 549–599, 2017.

[16] M. C. R. Alavanja, J. A. Hoppin, and F. Kamel, "Health effects of chronic pesticide exposure: cancer and neurotoxicity," *Annu. Rev. Public Heal.*, vol. 25, pp. 155–197, 2004.

[17] K. L. Bassil, C. Vakil, M. Sanborn, D. C. Cole, J. S. Kaur, and K. J. Kerr, "Cancer health effects of pesticides: systematic review," *Can. Fam. Physician*, vol. 53, no. 10, pp. 1704–1711, 2007.

[18] A. Blair and S. H. Zahm, "Agricultural exposures and cancer.," *Environ. Health Perspect.*, vol. 103, no. suppl 8, pp. 205–208, 1995.

[19] L. Horrigan, R. S. Lawrence, and P. Walker, "How sustainable agriculture can address the environmental and human health harms of industrial agriculture.," *Environ. Health Perspect.*, vol. 110, no. 5, pp. 445–456, 2002.

[20] R. W. Clapp, M. M. Jacobs, and E. L. Loechler, "Environmental and occupational causes of cancer: new evidence 2005-2007," *Rev. Environ. Health*, vol. 23, no. 1, pp. 1–38, 2008.

[21] N. Nazam, M. I. Lone, S. Shaikh, and W. Ahmad, "Assessment of genotoxic potential of the insecticide Dichlorvos using cytogenetic assay," *Interdiscip. Toxicol.*, vol. 6, no. 2, pp. 77–82, 2013.

[22] N. Nazam *et al.*, "Dimethoate induces dna damage and mitochondrial dysfunction triggering apoptosis in rat bone-marrow and peripheral blood cells," *Toxics*, vol. 8, no. 4, p. 80, 2020.

[23] M. Iqbal Lone, N. Nazam, S. Shaikh, and W. Ahmad, "Genotoxicity of an organochlorine pesticide dichlorophene by micronucleus and chromosomal aberration assays using bone marrow cells of Rattus norvegicus," *Caryologia*, vol. 66, no. 4, pp. 296–303, 2013.

[24] M. I. Lone *et al.*, "Genotoxicity and immunotoxic effects of 1, 2-dichloroethane in Wistar rats," *J. Environ. Sci. Heal. Part C*, vol. 34, no. 3, pp. 169–186, 2016.

[25] S. Mohafrash, E. Hassan, N. El-Shaer, and A. Mossa, "Detoxification gene expression, genotoxicity, and hepatorenal damage induced by subacute exposure to the new pyrethroid, imiprothrin, in rats," *Environ. Sci. Pollut. Res. Int.*, Jul. 2021, doi: 10.1007/S11356-021-13044-Z.

[26] A. T. H. Mossa, A. A. Refaie, A. Ramadan, and J. Bouajila, "Antimutagenic effect of Origanum majorana L. essential oil against prallethrin-induced genotoxic damage in rat bone marrow cells," *J. Med. Food*, vol. 16, no. 12, pp. 1101–1107, 2013, doi: 10.1089/jmf.2013.0006.

[27] A. A. Refaie, A. Ramadan, N. M. Sabry, W. K. B. Khalil, and A.-T. H. Mossa, "Over-gene expression in the apoptotic, oxidative damage and liver injure in female rats exposed to butralin," *Environ. Sci. Pollut. Res.*, vol. 27, no. 25, 2020,

doi: 10.1007/s11356-020-09416-6.
[28] M. F. Gad, H. R. Abdel-Rahman, and E. E.-S. Tawfeek, "Gene expression, DNA and kidney damage induced by pirimiphos-methyl in male mice: Chemical composition and ameliorative role of Origanum majorana leaves extract," *Egypt. J. Chem.*, vol. 63, no. 10, pp. 4141–4155, 2020.
[29] P. Peitl Jr, E. T. Sakamoto-Nojo, and I. M. Cólus, "Genotoxic activity of the insecticide Nuvacron (Monocrotophos) detected by the micronucleus test in bone marrow erythrocytes of mice and in CHO cells," *Brazilian J. Genet.*, vol. 19, pp. 571–576, 1996.
[30] O. A. Alabi, K. O. Ogunwenmo, T. T. Adebusuyi, and O. D. Shobowale, "Genotoxic potential of pirimiphos-methyl organophosphate pesticide using the mouse bone marrow erythrocyte micronucleus and the sperm morphology assay," *J. Environ. Occup. Heal.*, vol. 3, no. 2, pp. 81–86, 2014.
[31] I. Ayed-Boussema, K. Rjiba, N. Mnasri, A. Moussa, and H. Bacha, "Genotoxicity evaluation of dimethoate to experimental mice by micronucleus, chromosome aberration tests, and comet assay," *Int. J. Toxicol.*, vol. 31, no. 1, pp. 78–85, 2012.
[32] S. K. Yaduvanshi, N. Srivastava, F. Marotta, S. Jain, and H. Yadav, "Evaluation of micronuclei induction capacity and mutagenicity of organochlorine and organophosphate pesticides," *Drug Metab. Lett.*, vol. 6, no. 3, pp. 187–197, 2012.
[33] R. O. Sule, L. Condon, and A. V Gomes, "A common feature of pesticides: oxidative stress—the role of oxidative stress in pesticide-induced toxicity," *Oxid. Med. Cell. Longev.*, vol. 2022, 2022.
[34] A. M. Silva, C. Martins-Gomes, S. S. Ferreira, E. B. Souto, and T. Andreani, "Molecular physicochemical properties of selected pesticides as predictive factors for oxidative stress and apoptosis-dependent cell death in Caco-2 and HepG2 cells," *Int. J. Mol. Sci.*, vol. 23, no. 15, p. 8107, 2022.
[35] A. K. Aranda-Rivera, A. Cruz-Gregorio, Y. L. Arancibia-Hernández, E. Y. Hernández-Cruz, and J. Pedraza-Chaverri, "RONS and Oxidative Stress: An Overview of Basic Concepts," *Oxygen*, vol. 2, no. 4, pp. 437–478, 2022.
[36] K. Yoshikawa, K. Inagaki, T. Terashita, J. Shishiyama, S. Kuo, and D. M. Shankel, "Antimutagenic activity of extracts from Japanese eggplant," *Mutat. Res. Toxicol.*, vol. 371, no. 1–2, pp. 65–71, 1996.
[37] D. M. Maron and B. N. Ames, "Revised methods for the Salmonella mutagenicity test," *Mutat. Res. Mutagen. Relat. Subj.*, vol. 113, no. 3–4, pp. 173–215, 1983.
[38] H. T. Hoang, J. Y. Moon, and Y. C. Lee, "Natural antioxidants from plant extracts in skincare cosmetics: Recent applications, challenges and perspectives," *Cosmetics*, vol. 8, no. 4, p. 106, Nov. 2021, doi: 10.3390/cosmetics8040106.
[39] V. Lobo, A. Patil, A. Phatak, and N. Chandra, "Free radicals, antioxidants and functional foods: Impact on human health," *Pharmacogn. Rev.*, vol. 4, no. 8, pp. 118–126, Jul. 2010, doi: 10.4103/0973-7847.70902.
[40] J. Boubaker *et al.*, "Mutagenic, antimutagenic and antioxidant potency of leaf extracts from Nitraria retusa," *Food Chem. Toxicol.*, vol. 48, no. 8–9, pp. 2283–2290, Aug. 2010, doi: 10.1016/j.fct.2010.05.061.
[41] F. G. da S. Dantas, P. F. de Castilho, A. A. de Almeida-Apolonio, R. P. de Araújo, and K. M. P. de Oliveira, "Mutagenic potential of medicinal plants evaluated by the Ames Salmonella/microsome assay: A systematic review," *Mutat. Res. - Rev. Mutat. Res.*, vol. 786, Oct. 2020, doi: 10.1016/j.mrrev.2020.108338.
[42] V. Lattanzio, P. A. Kroon, V. Linsalata, and A. Cardinali, "Globe artichoke: A functional food and source of nutraceutical ingredients," *J. Funct. Foods*, vol. 1, no. 2, pp. 131–144, Apr. 2009, doi: 10.1016/j.jff.2009.01.002.
[43] L. López-Salas *et al.*, "Artichoke by-products as natural source of phenolic food ingredient," *Appl. Sci.*, vol. 11, no. 9, p. 3788, 2021.
[44] A.-I. Gostin and V. Y. Waisundara, "Edible flowers as functional food: A review on artichoke (Cynara cardunculus L.)," *Trends Food Sci. Technol.*, vol. 86, pp. 381–391, 2019.
[45] R. Jain and S. K. Jain, "Effect of Buchanania lanzan Spreng. bark extract on cyclophosphamide induced genotoxicity and oxidative stress in mice," *Asian Pac. J. Trop. Med.*, vol. 5, no. 3, pp. 187–191, Mar. 2012, doi: 10.1016/S1995-7645(12)60022-4.
[46] L. D. Brito *et al.*, "In vivo assessment of antioxidant, antigenotoxic, and antimutagenic effects of bark ethanolic extract from Spondias purpurea L," *J. Toxicol. Environ. Heal. - Part A Curr. Issues*, vol. 85, no. 8, pp. 336–352, 2022, doi: 10.1080/15287394.2021.2013373.
[47] C. de S. Araujo *et al.*, "Protective effects of bark ethanolic extract from spondias dulcis forst f. Against dna damage induced by benzo[a]pyrene and cyclophosphamide," *Genet. Mol. Biol.*, vol. 42, no. 3, pp. 643–654, Jul. 2019, doi: 10.1590/1678-4685-gmb-2018-0038.
[48] M. P. Kulikov, V. N. Statsenko, E. V. Prazdnova, and S. A. Emelyantsev, "Antioxidant, DNA-protective, and SOS inhibitory activities of Enterococcus durans metabolites," *Gene Reports*, vol. 27, Jun. 2022, doi: 10.1016/j.genrep.2022.101544.
[49] K. Słoczyńska, B. Powroźnik, E. Pękala, and A. M. Waszkielewicz, "Antimutagenic compounds and their possible mechanisms of action," *J. Appl. Genet.*, vol. 55, pp. 273–285, 2014.
[50] F. Pérez-García, T. Adzet, and S. Cañigueral, "Activity of artichoke leaf extract on reactive oxygen species in human leukocytes," *Free Radic. Res.*, vol. 33, no. 5, pp. 661–665, 2000, doi: 10.1080/10715760000301171.
[51] K. Rezazadeh, S. Aliashrafi, M. Asghari-Jafarabadi, and M. Ebrahimi-Mameghani, "Antioxidant response to artichoke

leaf extract supplementation in metabolic syndrome: A double-blind placebo-controlled randomized clinical trial," *Clin. Nutr.*, vol. 37, no. 3, pp. 790–796, Jun. 2018, doi: 10.1016/j.clnu.2017.03.017.

[52] K. Rezazadeh and M. Ebrahimi-Mameghani, "Artichoke leaf extract and use in metabolic syndrome as an antioxidant," in *Pathology: Oxidative Stress and Dietary Antioxidants*, Elsevier, 2020, pp. 169–177. doi: 10.1016/B978-0-12-815972-9.00016-0.

[53] G. C. Liao, J. H. Jhuang, and H. T. Yao, "Artichoke leaf extract supplementation lowers hepatic oxidative stress and inflammation and increases multidrug resistance-associated protein 2 in mice fed a high-fat and high-cholesterol diet," *Food Funct.*, vol. 12, no. 16, pp. 7239–7249, Aug. 2021, doi: 10.1039/d1fo00861g.

[54] A. T. H. Mossa, F. M. Ibrahim, S. M. M. Mohafrash, D. H. Abou Baker, and S. El Gengaihi, "Protective effect of ethanolic extract of grape pomace against the adverse effects of cypermethrin on weanling female rats," *Evidence-based Complement. Altern. Med.*, vol. 2015, 2015, doi: 10.1155/2015/381919.

[55] A. T. H. Mossa, M. A. Abdel Rasoul, and S. M. M. Mohafrash, "Lactational exposure to abamectin induced mortality and adverse biochemical and histopathological effects in suckling pups," *Environ. Sci. Pollut. Res.*, vol. 24, no. 11, pp. 10150–10165, Apr. 2017, doi: 10.1007/s11356-017-8600-x.

[56] A. T. H. Mossa and G. A. M. Nawwar, "Free radical scavenging and antiacetylcholinesterase activities of Origanum majorana L. essential oil," *Hum. Exp. Toxicol.*, vol. 30, no. 10, pp. 1501–1513, 2011, doi: 10.1177/0960327110391686.

[57] A. H. Mossa, T. M. Heikal, M. Belaiba, E. G. Raoelison, H. Ferhout, and J. Bouajila, "Antioxidant activity and hepatoprotective potential of Cedrelopsis grevei on cypermethrin induced oxidative stress and liver damage in male mice," *BMC Complement. Altern. Med.*, vol. 15, no. 1, 2015, doi: 10.1186/s12906-015-0740-2.

[58] NRC, "Guide for the care and use of laboratory animals," *Counc. Natl. Res.*, 2010.

[59] C. D. S. Tomlin, *The e-Pesticides Manual," Version 3.0, 13th ed., BCPC (British Crop Protection Council)*. 2004.

[60] I. D. Adler, "Cytogenetic tests in mammals," *Mutagen. testing-A Pract. approach*, 1984.

[61] OECD, *Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test*. 2014. doi: 10.1787/9789264224407-en.

[62] W. Schmid, "The micronucleus test," *Mutat. Res. Mutagen. Relat. Subj.*, vol. 31, no. 1, pp. 9–15, 1975, doi: 10.1016/0165-1161(75)90058-8.

[63] U. J. A. D'Souza, A. Zain, and S. Raju, "Genotoxic and cytotoxic effects in the bone marrow of rats exposed to a low dose of paraquat via the dermal route," *Mutat. Res. - Genet. Toxicol. Environ. Mutagen.*, vol. 581, no. 1–2, pp. 187–190, Mar. 2005, doi: 10.1016/j.mrgentox.2004.10.019.

[64] OECD, "Guideline 474: Mammalian Erythrocyte Micronucleus Test," *Ocde*, no. July, pp. 1–21, Jul. 2016, doi: 10.1787/9789264264762-EN.

[65] M. Tudi *et al.*, "Agriculture development, pesticide application and its impact on the environment," *Int. J. Environ. Res. Public Health*, vol. 18, no. 3, p. 1112, 2021.

[66] G. Pizzino *et al.*, "Oxidative Stress: Harms and Benefits for Human Health," *Oxid. Med. Cell. Longev.*, vol. 2017, 2017, doi: 10.1155/2017/8416763.

[67] J. Q. Wu, T. R. Kosten, and X. Y. Zhang, "Free radicals, antioxidant defense systems, and schizophrenia," *Prog. Neuro-Psychopharmacology Biol. Psychiatry*, vol. 46, pp. 200–206, 2013.

[68] S. Baliyan *et al.*, "Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa," *Molecules*, vol. 27, no. 4, Feb. 2022, doi: 10.3390/molecules27041326.

[69] F. Shahidi and Y. Zhong, "Measurement of antioxidant activity," *J. Funct. Foods*, vol. 18, pp. 757–781, Oct. 2015, doi: 10.1016/j.jff.2015.01.047.

[70] N. Jiménez-Moreno *et al.*, "Phenolic composition of artichoke waste and its antioxidant capacity on differentiated Caco-2 cells," *Nutrients*, vol. 11, no. 8, p. 1723, 2019.

[71] H. Falleh *et al.*, "Phenolic composition of Cynara cardunculus L. organs, and their biological activities," *C. R. Biol.*, vol. 331, no. 5, pp. 372–379, 2008.

[72] W. Biel, R. Witkowicz, E. Piątkowska, and C. Podsiadło, "Proximate composition, minerals and antioxidant activity of artichoke leaf extracts," *Biol. Trace Elem. Res.*, vol. 194, pp. 589–595, 2020.

[73] O. L. Erukainure, O. Sanni, and M. S. Islam, "Clerodendrum volubile: phenolics and applications to health," in *Polyphenols: Mechanisms of action in human health and disease*, Elsevier, 2018, pp. 53–68.

[74] S. K. Shebeko, I. A. Zupanets, O. S. Popov, O. O. Tarasenko, and A. S. Shalamay, "Polyphenols: Mechanisms of Action in Human Health and Disease." Academic Press Cambridge, MA, USA:, 2018.

[75] M. F. Matei, R. Jaiswal, and N. Kuhnert, "Investigating the chemical changes of chlorogenic acids during coffee brewing: Conjugate addition of water to the olefinic moiety of chlorogenic acids and their quinides," *J. Agric. Food Chem.*, vol. 60, no. 49, pp. 12105–12115, 2012.

[76] N. Liang and D. D. Kitts, "Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions," *Nutrients*, vol. 8, no. 1, p. 16, 2015.

[77] F. Mastorci, N. Linzalone, L. Ait-Ali, and A. Pingitore, "Environment in children's health: a new challenge for risk assessment," *Int. J. Environ. Res. Public Health*, vol. 18, no. 19, p. 10445, 2021.

[78] WHO, "Summary of principles for evaluating health risks in children associated with exposure to chemicals," 2011.

[79] WHO, “Healthy environments for children-initiating an alliance for action.” World Health Organization Geneva, 2002.
[80] WHO, “Initiating an Alliance for Action: Healthy Environments for Children.” World Health Organization Geneva, 2002.
[81] P. Winchester, C. Proctor, and J. Ying, “County-level pesticide use and risk of shortened gestation and preterm birth,” *Acta Paediatr.*, vol. 105, no. 3, pp. e107–e115, 2016.
[82] L. L. De Zwart, H. Haenen, C. H. M. Versantvoort, and A. Sips, “Pharmacokinetics of ingested xenobiotics in children: A comparison with adults,” 2002.
[83] N. H. Perlroth and C. W. C. Branco, “O estado atual do conhecimento sobre a exposição ambiental no organismo infantil durante os períodos sensíveis de desenvolvimento☆,” *J. Pediatr. (Rio. J).*, vol. 93, pp. 17–27, 2017.
[84] M. J. S. Treviño *et al.*, “How pesticides affect neonates?-Exposure, health implications and determination of metabolites,” *Sci. Total Environ.*, vol. 856, p. 158859, 2023.
[85] S. H. Zahm and M. H. Ward, “Pesticides and childhood cancer.,” *Environ. Health Perspect.*, vol. 106, no. suppl 3, pp. 893–908, 1998.
[86] J. Jurewicz and W. Hanke, “Exposure to pesticides and childhood cancer risk: has there been any progress in epidemiological studies?,” *Int. J. Occup. Med. Environ. Health*, vol. 19, no. 3, p. 152, 2006.
[87] V. S. Melanda, M. E. A. Galiciolli, L. S. Lima, B. C. Figueiredo, and C. S. Oliveira, “Impact of Pesticides on Cancer and Congenital Malformation: A Systematic Review,” *Toxics*, vol. 10, no. 11, p. 676, 2022.
[88] F. A. Sloan and H. Gelband, “Cancer causes and risk factors and the elements of cancer control,” in *Cancer Control Opportunities in Low-and Middle-Income Countries*, National Academies Press (US), 2007.
[89] M. Bruce, “A systematic and conceptual review of posttraumatic stress in childhood cancer survivors and their parents,” *Clin. Psychol. Rev.*, vol. 26, no. 3, pp. 233–256, 2006.
[90] J. L. Daniels, A. F. Olshan, and D. A. Savitz, “Pesticides and childhood cancers.,” *Environ. Health Perspect.*, vol. 105, no. 10, pp. 1068–1077, 1997.
[91] E. L. Robb and M. B. Baker, “Organophosphate toxicity,” 2017.
[92] R. Kamanyire and L. Karalliedde, “Organophosphate toxicity and occupational exposure,” *Occup. Med. (Chic. Ill).*, vol. 54, no. 2, pp. 69–75, 2004.
[93] N. Comfort and D. B. Re, “Sex-specific neurotoxic effects of organophosphate pesticides across the life course,” *Curr. Environ. Heal. reports*, vol. 4, pp. 392–404, 2017.
[94] T. A. Kato and J. S. Haskins, “Mitotic index analysis,” in *Chromosome Analysis: Methods and Protocols*, Springer, 2022, pp. 17–26.
[95] M. Inoue, M. W. Miller, C. Cox, and E. L. Carstesen, “Growth rate and mitotic index analysis of Vicia faba L. Roots exposed to 60-Hz electric fields,” *Bioelectromagn. J. Bioelectromagn. Soc. Soc. Phys. Regul. Biol. Med. Eur. Bioelectromagn. Assoc.*, vol. 6, no. 3, pp. 293–303, 1985.
[96] S. A. Salazar Mercado and J. D. Quintero Caleño, “Determination of malathion’s toxic effect on Lens culinaris Medik cell cycle,” *Heliyon*, vol. 6, no. 9, p. e04846, Sep. 2020, doi: 10.1016/j.heliyon.2020.e04846.
[97] E. Renieri, E. Vakonaki, and P. Fragkiadaki, “Chromosome aberrations,” in *Reference Module in Biomedical Sciences*, Elsevier, 2023. doi: 10.1016/b978-0-12-824315-2.00738-7.
[98] P. Lovreglio *et al.*, “Evaluation of chromosome aberration and micronucleus frequencies in blood lymphocytes of workers exposed to low concentrations of benzene,” *Mutat. Res. - Genet. Toxicol. Environ. Mutagen.*, vol. 770, pp. 55–60, 2014, doi: 10.1016/j.mrgentox.2014.04.022.
[99] K. Hirschhorn and H. L. Cooper, “Chromosomal aberrations in human disease. A review of the status of cytogenetics in medicine,” *The American Journal of Medicine*, vol. 31, no. 3. Elsevier, pp. 442–470, Sep. 01, 1961. doi: 10.1016/0002-9343(61)90128-0.
[100] T. Just, E. W. Sinnott, and L. C. Dunn, “Principles of Genetics.,” *Am. Midl. Nat.*, vol. 22, no. 3, p. 755, 1939, doi: 10.2307/2420356.
[101] F. N. Dulout *et al.*, “Sister-chromatid exchanges and chromosomal aberrations in a population exposed to pesticides,” *Mutat. Res. Lett.*, vol. 143, no. 4, pp. 237–244, 1985.
[102] S. M. Bréga *et al.*, “Clinical, cytogenetic and toxicological studies in rural workers exposed to pesticides in Botucatu, São Paulo, Brazil,” *Cad. Saude Publica*, vol. 14, no. suppl 3, pp. S117–S123, 1998.
[103] B.-W. Igl *et al.*, “The rat bone marrow micronucleus test: Statistical considerations on historical negative control data,” *Regul. Toxicol. Pharmacol.*, vol. 102, pp. 13–22, 2019.
[104] C. Bolognesi, A. Creus, P. Ostrosky-Wegman, and R. Marcos, “Micronuclei and pesticide exposure,” *Mutagenesis*, vol. 26, no. 1, pp. 19–26, 2011.
[105] C. Bolognesi, M. Parrini, F. Merlo, and S. Bonassi, “Frequency of micronuclei in lymphocytes from a group of floriculturists exposed to pesticides,” *J. Toxicol. Environ. Heal. Part A Curr. Issues*, vol. 40, no. 2–3, pp. 405–411, 1993.
[106] S. Pastor *et al.*, “Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers,” *Mutagenesis*, vol. 18, no. 3, pp. 249–258, 2003.

[107] C. G. Torquetti, A. T. B. Guimarães, and B. Soto-Blanco, "Exposure to pesticides in bats," *Sci. Total Environ.*, vol. 755, p. 142509, 2021.

[108] K. Kaur and R. Kaur, "Occupational pesticide exposure, impaired DNA repair, and diseases," *Indian J. Occup. Environ. Med.*, vol. 22, no. 2, p. 74, 2018.

[109] X. Lu and C. Yu, "Enantiomer-specific profenofos-induced cytotoxicity and DNA damage mediated by oxidative stress in rat adrenal pheochromocytoma (PC12) cells," *J. Appl. Toxicol.*, vol. 34, no. 2, pp. 166–175, Feb. 2014, doi: 10.1002/jat.2847.

[110] A. K. Pandey, N. S. Nagpure, and S. P. Trivedi, "Genotoxicity assessment of pesticide profenofos in freshwater fish Channa punctatus (Bloch) using comet assay and random amplified polymorphic DNA (RAPD)," *Chemosphere*, vol. 211, pp. 316–323, Nov. 2018, doi: 10.1016/j.chemosphere.2018.07.182.

[111] X. Lihui, Q. Xiaojie, Y. Hao, C. Jialiang, G. Jinming, and C. Ying, "Albicanol modulates oxidative stress and the p53 axis to suppress profenofos induced genotoxicity in grass carp hepatocytes," *Fish Shellfish Immunol.*, vol. 122, pp. 325–333, Mar. 2022, doi: 10.1016/j.fsi.2022.02.002.

[112] S. Shadnia *et al.*, "Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators," *Hum. Exp. Toxicol.*, vol. 24, no. 9, pp. 439–445, 2005.

[113] M. Abdollahi, A. Jafari, and N. Jalali, "Chronic toxicity in organophosphate exposed workers," *MJIRI*, vol. 9, pp. 221–225, 1995.

[114] N. Loganayaki, P. Siddhuraju, and S. Manian, "Antioxidant activity and free radical scavenging capacity of phenolic extracts from Helicteres isora L. and Ceiba pentandra L.," *J. Food Sci. Technol.*, vol. 50, pp. 687–695, 2013.

[115] N. Ceccarelli, M. Curadi, P. Picciarelli, L. Martelloni, C. Sbrana, and M. Giovannetti, "Globe artichoke as a functional food," *Med. J. Nutrition Metab.*, vol. 3, no. 3, pp. 197–201, 2010.

[116] R. Gebhardt, "Anticholestatic activity of flavonoids from artichoke (Cynara scolymus L.) and of their metabolites.," *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.*, vol. 7, pp. 316–320, 2001.

[117] G. B. Mahady, "Medicinal plants for the prevention and treatment of coronary heart disease," *Ethnopharmacology*, vol. 2, pp. 75–99, 2009.