Thiamethoxam-induced hematological, biochemical, and genetic alterations and the ameliorative effect of *Moringa oleifera* in male mice

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**A B S T R A C T**

Thiamethoxam (TMX) exerts pronounced insecticidal effects against a wide variety of economically imperative pests. However, the administration of TMX in experimental animals induced notable adverse effects on the function of various organs. The purpose of this study was to assess TMX induced hematological, biochemical, and genetic alterations and the potential ameliorative effects on them of *Moringa oleifera* leaf extract (MLE) in male mice. Animals were orally administered TMX (≈1/10 LD_{50}) daily either alone or with MLE (200 mg/kg b.w.) for 28 successive days. Blood was collected to evaluate the hematological profile and serum levels of Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, creatinine, uric acid, and urea. Liver and kidney cells were used to assess the Malondialdehyde (MDA) content and antioxidant enzymes. DNA integrity was estimated also in the liver and kidney using comet and colorimetric diphenylamine assays. Results revealed that TMX exhibited significant changes in the hematological profile and liver and kidney functions. Besides, TMX significantly raised the MDA content and DNA damage in both two of these organs. In contrast, TMX reduced the antioxidant activities in the cells of both liver and kidney. Meanwhile, Moringa extract combined with TMX significantly attenuated the deleterious findings of TMX. Specifically, it improved the TMX-induced hematological changes, liver and kidney function alterations, oxidative stress, and DNA damage rate. It can be concluded that TMX had adverse effects on different cells of male mice, but MLE successfully ameliorated TMX’s hematological and hepatorenal toxicity.

1. Introduction

Pesticides are chemicals introduced into the environment that affect crop production and food grain storage by eradicating vectors associated with disease transmission [1]. Recently, pesticides have been extensively utilized to enhance crop production. However, health hazards arising from anthropogenic exposure to these chemicals have become a serious concern [2].

Pesticides are typically taken up by farmers via respiration, ingestion, and cutaneous, and then circulate through the blood affecting various organs. The liver and kidneys are the main organs participating in the process of detoxification through the biotransformation and excretion of xenobiotics and their metabolites, so these organs are highly susceptible to injury by xenobiotics [1,3]. The hepatotoxicity and nephrotoxicity of pesticides have been proven by research in experimental animals [4,5].

Thiamethoxam (TMX), (EZ)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5 oxadi-azinan-4-ylidene (nitro) amine (IUPAC), is a second-generation neonicotinoid with high insecticidal efficacy; it is used against a variety of economically important pests [6]. TMX was found to act as a biomarker of hepatotoxicity, which is possibly associated with histological lesions, tumor growth, and cell death in mice [7]. More recently, some hematobiochemical studies confirmed that anemia and alterations of liver and kidney biomarkers were reliable findings in TMX treated cockerels [8], birds [9–11], fish [12,13], and rats [14–16]. Besides, the mutagenic effect of TMX has been demonstrated in many studies [17,18].

In all civilizations and societies, plant-derived drugs have been widely utilized and played a vital role in healthcare. The initial modes of herbal treatment are a section of society and the strategy of principal therapy in most developing nations. These alternative remedies are socially accepted, cheap, readily available, and often effective [19].

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Moringa plant has nutritional and medicinal properties [20]. Given its therapeutic uses and nutritional benefits, it has great economic importance [21]. It is a member of the Moringaceae family, which is indigenous to India, Bangladesh, Pakistan, and Afghanistan in the sub-Himalayan zones [22]. In addition to the various minerals present in *Moringa* leaves and seeds, *M. oleifera* is rich in various other active phytoconstituents (tannins, steroids, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, and vitamins). The existence of these components is responsible for this plant’s antioxidant effects and the ability to defend against free radicals [23–25]. Kaempferol, quercetin, and chlorogenic acid, which have antiproliferative, anti-inflammatory, and antihypertensive properties, are the major polyphenols present in *Moringa* extracts [20,26,27]. In addition, *Moringa oleifera* leaf extract (MLE) was shown to successfully protect against liver injury induced by several toxins [28,29].

The aim of this research was to evaluate the toxicity of TMX and the alleviative role of MLE against the deleterious effects of TMX in male mice. To achieve this, hematological analysis, estimations of liver and kidney function, assessments of oxidative stress and antioxidant activities, and DNA damage evaluation were carried out.

2. Materials and methods

2.1. Chemicals

The insecticide thiamethoxam (TMX) (≥98.0 %) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of *Moringa oleifera* leaf extract

The leaves of *Moringa oleifera* were collected from the farm of the Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University. The leaves were cleaned, cut into small pieces, and dried in the shade. The dry leaves were then crushed using an electrical homogenizer. The MLE was prepared by adding 1 g of powdered leaves to 10 mL of boiling water for 5 min, in accordance with the procedure reported by Berkovich et al. [30].

2.3. Animals

Forty male Swiss albino mice aged 10 weeks old and 26 ± 5 g in weight were purchased from the Institute of Theodor Bilharz, Giza, Egypt. The animals were kept in polypolyrene cages with stainless steel mesh covers in the animal house of the Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University. The animals were supplied with standard animal pellets and water *ad libitum*, maintained under controlled temperature (23 °C ± 4 °C), 50–55 % relative humidity, and a 12 h light:12 h dark cycle, and were allowable to acclimate for 2 weeks prior to the start of the experiments. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication 86–23, revised 1985). The protocol was approved by the Ethics Committee of the National Research Centre (Approval No. 20/132).

2.4. Treatment schedule

The animals were divided into four groups, each containing ten animals with almost equal body weights, and dosed orally as follows: Group I: animals were given distilled water and served as a control; Group II: animals were given MLE at a dose of 200 mg/kg b.w. for 28 days in accordance with the procedure of Salama et al. [31]; Group III: animals were given an oral dose of TMX (≈1/10 LD50 = 87.73 mg TMX/kg b.w. for 28 days), in line with the procedure of Saadi et al. [32]; and Group IV: animals were orally administered MLE plus TMX for 28 days in the same manner as in the second and third groups.

2.5. Determination of relative weights of organs

At the end of the treatment period, mice were sacrificed by cervical decapitation. The liver and kidneys were dissected out; extra fat was removed, and the relative organ weight percentages were calculated as follows:

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\text{The relative weight of organ %} = \frac{\text{Absolute organ weight (g)}}{\text{Final body weight (g)}} \times 100
\]

2.6. Sample collection

The animals were fasted for 8 h after the experimental phase, until blood samples were taken. Blood was drawn from the orbital venous retrograde plexus of the eye using capillary tubing, collected in clean tubes, and split into two sections. The first section was utilized for hematological analysis with heparin as an anticoagulant (1-2 IU/ mL). The second part was left to coagulate for about 20 min at room temperature and then centrifuged for 10 min at 3000 rpm. To estimate liver and kidney function parameters, the serum was isolated and stored at 20 °C in an Eppendorf tube. The mice were killed by cervical dislocation after obtaining the blood samples, and their livers and kidneys were immediately removed and kept at 4 °C for use in genetic and oxidative stress analyses.

2.7. Hematological analysis

The erythrocyte count (EC), platelet count (Plt), and leukocyte count (LC) were calculated using a hematology analyzer (Medonic C620/530 Vet), while the hemoglobin (Hb) level was determined according to the process reported by Vankampen and Zylstra [33].

2.8. Liver and kidney function biomarkers

In accordance with the method of Reitman and Frankel [34], ALT and AST were assessed using a biodiagnostic kit (Cat. No. AT 10 34 45) and ALP was analyzed by the method of Belfield and Goldberg [35] with a diagnostic kit (Cat. No. AP 10 20) from Biodiagnostic Co. (Egypt). Albumin was measured following the method of Doumas et al. [36] using a kit (Cat. No. AB 10 10). The methods of Patton and Crouch [37] and Henry et al. [38] were used to determine kidney function, including urea and creatinine levels. Urine acid was also measured in line with the method of Fossati et al. [39]. All determinations were calorimetrically measured by a spectrophotometer using assay kits (Diamond Diagnostics, Egypt).

2.9. Tissue homogenate preparation

The liver and kidney tissues were isolated, excised, and rinsed in chilled saline (0.9 % w/v NaCl) and homogenate using a Potter-Elvehjem homogenizer in chilled phosphate buffer (0.1 M, pH 7.4). The homogenate was passed through a muslin cloth and centrifuged to separate the nuclear debris in a cooled centrifuge at 4 °C and 1000 rpm for 5 min. Supernatants were kept in a deep freezer at -20 °C until they were used for biochemical and genetical analyses.

2.10. Assessment of oxidative stress and enzymatic antioxidants

As described by Ohikawa et al. [40], lipid peroxidation was evaluated by measuring the MDA level. Thiobarbituric acid reacted with MDA in acidic medium at a temperature of 95 °C for 30 min, resulting in a pink product. The absorbance of the product could be measured at 534 nm.

Reduced glutathione (GSH) was measured in line with the process reported by Beutler et al. [41]. The method based on the reduction of 5, 5’-dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) was used to produce a yellow compound. The reduced chromogen was
directly proportional to the GSH concentration and its absorbance could be measured at 405 nm.

Catalase (CAT) activity was estimated as described by Aebi [42]. Catalase reacts with a known quantity of H$_2$O$_2$. The reaction was stopped after exactly 1 min using a catalase inhibitor. In the presence of peroxidase (HRP), the remaining H$_2$O$_2$ reacted with 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample, as measured at 510 nm.

Assessment of the activity of superoxide dismutase (SOD) was performed based on the ability of the enzyme to inhibit the phenazine methosulfate-mediated reduction of nitroblue tetrazolium dye. The increase in absorbance was measured at 560 nm, in accordance with the procedure of Nishikimi et al. [43].

2.11. Genotoxicity and antigenotoxicity assays

2.11.1. DNA damage by comet assay

In accordance with the work of Elhamalawy and El Makawy [44], DNA damage was determined in liver and kidney cells. On pre-coated microscopic slides with a regular melting point, agarose cells were loaded. The drying slides were placed in a (pH 10) lysis buffer (1% Triton X-100, 2.5 M NaCl, 10 mM Tris HCl, 0.1 M EDTA) at 4°C for at least 2 h to lyse the cells and separate the DNA from the histones. The DNA was unwound in a chilled alkaline solution (0.3 M NaOH, 1 mM EDTA) for 40 min and electrophoresed for 20 min (25 V, 300 mA electrophoresis platform). Neutralization was achieved in 0.4 M Tris buffer (pH 7.5) for 10 min and H$_2$O for 5 min. Gels were dehydrated, fixed with methanol for 5 min, and then stained with ethidium bromide in TAE buffer (1 μg/mL). Fifty randomly chosen nuclei per trial were photographed using a fluorescence microscope (Eclipse 800; Nikon, Tokyo, Japan) and investigated using image analysis software (Comet Assay IV; Perceptive Instruments, Bury St Edmunds, UK).

2.11.2. DNA fragmentation by diphenylamine (DPA) assay

The detection of DNA fragmentation by DPA was performed in accordance with the procedure of Mutawakil et al. [45]. The homogenized tissues were lysed in 0.5 mL of lysis buffer containing 10 mM Tris-HCl (pH 8), 1 mM EDTA, and 0.2% Triton X-100, then centrifuged at 10,000 rpm for 15 min at 4°C. For protein elimination, the pellets were dissolved in 0.5 N perchloric acid and 5.5 N perchloric acid was added to the supernatants, followed by incubation at 90°C for 20 min and centrifugation for 10 min. Next, DPA solution was added and left at room temperature overnight. The optical density (OD) was measured at 600 nm by a spectrophotometer (Shimadzu UV 160 A; Shimadzu Co., Japan). The rate of fragmented DNA was determined as follows: OD of fragmented DNA (supernatant) / (OD of fragmented DNA (supernatant) + OD of intact DNA (pellet)) x 100.

2.12. Statistical analysis

Statistical analyses were performed using SPSS 16 software (SPSS Inc., Chicago, IL, USA). Experimental data were analyzed using one-way analysis of variance. The significance of differences between means was evaluated using Duncan’s multiple range test. All values are expressed as mean ± SE and the differences were considered significant at the $P \leq 0.05$ level.

3. Results

In this experiment, MLE (200 mg/kg) did not have any deleterious effects on the exposed animals. There were no significant variations in the weights of the liver and kidneys. The hematological parameters revealed that the extract did not induce change in the hematopoietic system. Furthermore, almost all of the biochemical indicators remained unchanged. Besides, no notable variations were observed in the DNA damage rate as measured by comet assay and DPA colorimetric analysis in the liver and kidney cells of MLE-administered animals.

3.1. Relative weights of organs

The oral administration of TMX (1/10 LD$_{50}$) to male mice for 28 consecutive days caused a significant increase ($P \leq 0.05$) in the relative liver (3.42 ± 0.02) and kidney (1.63 ± 0.02) weights relative to those in the control (3.32 ± 0.01 and 1.57 ± 0.02, respectively) (Fig. 1). MLE (200 mg/kg b.w.) concurrently administered with TMX significantly ($P \leq 0.05$) restored the relative liver (3.33 ± 0.02) and kidney (1.158 ± 0.01) weights to near normalcy compared with the findings in TMX-treated mice (Fig. 1).

3.2. Hematological parameters

The effects of TMX exposure and the modulatory effects of MLE on the hematological indices are presented in Table 1. The results clearly showed that TMX administration produced significant reductions ($P \leq 0.05$) in EC (4.23 ± 0.16), hemoglobin concentration (11.35 ± 0.27), and platelet count (192.33 ± 9.26) in comparison with those in control animals (5.49 ± 0.21, 15.68 ± 0.41, and 342.00 ± 10.44, respectively). In contrast, TMX induced a significant increase ($P \leq 0.05$) in LC (13.27 ± 0.31) compared with that in the control (6.60 ± 0.22), which may reflect activation of the mouse immune system. Combined gavage of MLE and TMX significantly ($P \leq 0.05$) reversed the changes in hematological measurements (EC: 4.95 ± 0.67, Hb: 14.64 ± 0.25, PL: 285.66 ± 7.13, and LC: 9.55 ± 0.21) compared with those in the TMX-treated group (4.23 ± 0.16, 11.35 ± 0.27, 192.33 ± 9.26, and 13.27 ± 0.31, respectively).

3.3. Biochemical analysis

3.3.1. Oxidative stress markers

As shown in Table 2, male mice treated with TMX exhibited significant ($P \leq 0.05$) increases in liver and kidney MDA levels (9.89 ± 0.28 and 8.34 ± 0.19, respectively) compared with the controls (5.35 ± 0.29 and 4.02 ± 0.24, respectively). Nevertheless, the levels of liver and kidney SOD (7.76 ± 0.57 and 6.73 ± 0.31), GSH (3.82 ± 0.23 and 2.83 ± 0.18), and CAT (9.39 ± 0.45 and 8.44 ± 0.28) were significantly ($P \leq 0.05$) diminished. Regarding the administration of TMX and MLE to male mice, MLE significantly ($P \leq 0.05$) attenuated the changes in liver and kidney MDA and increased the antioxidant enzymes compared with the findings in TMX-treated mice.

3.3.2. Liver function evaluation

Significant elevations in ALT (42.80 ± 1.88), AST (48.20 ± 1.16), and ALP (119.20 ± 2.37) accompanied by a significant decrease ($P \leq 0.05$)
Table 1: Effects of thiamethoxam (TMX) and/or Moringa leaf aqueous extract (MLE) on hematological parameters of male mice.

| Treatments     | EC (10^6/μL) | Hb (g/dL) | Pt (10^9/μL) | LC (10^9/μL) |
|----------------|--------------|-----------|--------------|--------------|
| Control        | 5.49 ± 0.21^a| 15.68 ± 0.41^a| 342.00 ± 10.44^a| 6.60 ± 0.22^a|
| MLE            | 5.65 ± 0.15^a| 16.34 ± 0.26^a| 357.67 ± 12.44^a| 6.96 ± 0.17^a|
| TMX            | 4.23 ± 0.16^a| 11.35 ± 0.27^a| 192.33 ± 9.26^a| 13.27 ± 0.31^a|
| TMX + MLE      | 4.95 ± 0.67^a| 14.64 ± 0.23^a| 285.66 ± 7.13^a| 9.55 ± 0.21^b|

Data are presented as mean ± SE. Different superscripts indicate significant differences within the same column (P ≤ 0.05) (EC: erythrocyte count, Hb: hemoglobin, Pt: platelets, LC: leukocyte count).

0.05) in albumin (3.79 ± 0.06) were observed in mice that received TMX (1/10 LD₅₀) for 28 consecutive days, compared with the findings in control animals (23.75 ± 1.11, 28.25 ± 1.10, 57.50 ± 1.55, and 4.49 ± 0.16), as shown in Table 3. Meanwhile, the levels of ALT, AST, ALP, and albumin in mice treated with both MLE and TMX were significantly changed (P ≤ 0.05) relative to those of mice given TMX alone.

3.3.3. Kidney function evaluation

As summarized in Table 4, TMX resulted in significant increases in the concentrations of creatinine (2.51 ± 0.02), urea (47.00 ± 1.52), and uric acid (7.56 ± 0.21) compared with those of control animals (0.61 ± 0.03, 23.75 ± 1.80, and 3.88 ± 0.20, respectively). In contrast, the administration of MLE plus TMX to male mice significantly (P ≤ 0.05) alleviated the excess levels of creatinine (1.38 ± 0.02), urea (28.80 ± 1.32), and uric acid (4.66 ± 0.23) of TMX-treated animals.

3.4. Genetic investigation

The damaged DNA in the liver and kidney was assessed by the comet assay and DPA colorimetric test.

3.4.1. Comet assay in liver and kidney cells

The mean values of comet parameters in the liver and kidneys of mice treated with TMX and/or MLE are recorded in Tables 5 & 6. TMX induced significant elevations (P ≤ 0.05) in the count of tail cells, the measured tail length, and the percentage of DNA in the tails, and olive tail moment in both cells of the two organs compared with the findings in the negative control. In contrast, MLE applied concurrently with TMX by gavage significantly (P ≤ 0.05) ameliorated the excess levels of the parameters as measured by the comet assay in liver and kidney cells of TMX-treated animals (Fig. 2).

3.4.2. DNA fragmentation by diphenylamine (DPA)

As shown in Fig. 3, TMX treatment significantly increased (P ≤ 0.05) the DNA fragmentation rates in liver (10.50 ± 0.25) and kidney (7.42 ± 0.25) cells compared with the findings in TMX-treated mice.

4. Discussion

Thiamethoxam is a neonicotinoid insecticide commonly used in agriculture, which is having an increasing impact on the environment. The purpose of this work was to explore whether variations in the

Table 3: Effects of thiamethoxam (TMX) and/or Moringa leaf aqueous extract (MLE) on the liver function of male mice.

| Treatments     | Albumin (mg/dL) | ALT (U/L) | AST (U/L) | ALP (U/L) |
|----------------|-----------------|-----------|-----------|-----------|
| Control        | 4.49 ± 0.16^a   | 23.75 ± 1.11^b| 28.25 ± 1.89^b| 57.50 ± 1.55^b|
| MLE            | 4.58 ± 0.04^a   | 24.75 ± 1.20^b| 27.00 ± 1.89^b| 55.25 ± 1.89^b|
| TMX            | 3.79 ± 0.96^a   | 42.80 ± 1.88^b| 48.20 ± 1.50^b| 119.20 ± 2.10^b|
| TMX + MLE      | 4.17 ± 0.06^a   | 30.20 ± 1.50^b| 32.40 ± 1.20^b| 82.40 ± 1.63^b|

Data are presented as mean ± SE. Significant differences (P ≤ 0.05) are indicated by different superscripts within the same column. AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase.

Table 4: Effects of thiamethoxam (TMX) and/or Moringa leaf aqueous extract (MLE) on the kidney function of male mice.

| Treatments     | Creatinine (mg/dL) | Urea (mg/dL) | Uric acid (mg/dL) |
|----------------|---------------------|--------------|-------------------|
| Control        | 0.61 ± 0.03^a       | 23.75 ± 1.80^b| 3.88 ± 0.20^b     |
| MLE            | 0.56 ± 0.02^a       | 22.00 ± 1.83^b| 3.15 ± 0.17^b     |
| TMX            | 2.51 ± 0.02^a       | 47.00 ± 1.52^b| 7.56 ± 0.21^b     |
| TMX + MLE      | 1.38 ± 0.02^a       | 28.80 ± 1.32^b| 4.66 ± 0.23^b     |

Data are expressed as mean ± SE. Different superscripts within the same column indicate significant differences (P ≤ 0.05).

Table 5: Impact of thiamethoxam (TMX) and/or Moringa leaf aqueous extract (MLE) on comet assay measurements in male mouse hepatocytes.

| Treatments     | Tail cells (%) | Tail length (μm) | Tail DNA (%) | Olive tail moment (%) |
|----------------|----------------|------------------|--------------|-----------------------|
| Control        | 8.67 ± 0.67^a  | 8.60 ± 0.38^a    | 10.75 ± 0.34^a| 0.93 ± 0.06^a         |
| MLE            | 7.33 ± 0.88^a  | 7.58 ± 0.41^a    | 10.40 ± 0.36^a| 0.79 ± 0.04^a         |
| TMX            | 34.33 ± 2.04^a | 20.44 ± 0.61^a   | 23.36 ± 0.22^a| 4.76 ± 0.18^a         |
| TMX + MLE      | 14.67 ± 1.45^b | 12.79 ± 0.51^b   | 15.62 ± 0.55^b| 2.00 ± 0.14^a         |

Data are shown as mean ± SE. Different superscripts in the same column represent significant differences (P ≤ 0.05).

Table 2: Effects of thiamethoxam (TMX) or/and Moringa leaf aqueous extract (MLE) on oxidative stress markers in mouse hepatic and renal homogenate.

| Treatments     | MDA mmol/mg | SOD U/mg | CAT U/mg | GSH mmol/mg |
|----------------|-------------|----------|----------|-------------|
|                | Hepatic     | Renal    | Hepatic  | Renal       |
| Control        | 5.35 ± 0.29^a| 4.02 ± 0.24^a| 13.52 ± 0.23^a| 12.56 ± 0.24^a|
| MLE            | 4.82 ± 0.20^a| 4.05 ± 0.14^a| 14.41 ± 0.34^a| 12.87 ± 0.39^a|
| TMX            | 9.89 ± 0.21^a| 8.34 ± 0.19^a| 7.76 ± 0.57^a| 6.73 ± 0.31^a|
| TMX + MLE      | 6.58 ± 0.16^a| 5.55 ± 0.22^a| 11.71 ± 0.40^a| 10.36 ± 0.55^a|

Data are presented as mean ± SE. Different superscripts indicate significant differences within the same column (P ≤ 0.05) (MDA: malondialdehyde, GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase).
hematology, oxidative stress, DNA integrity, and hepatic and renal function triggered by TMX could be alleviated by MLE. Significant increases in relative liver and kidney weights were noted in male mice treated with TMX. These increases may have been due to xenobiotic-associated injuries [46]. The different biomarkers assessed in the blood are excellent indicators for various physiological and pathological changes in animal and human health [47]. The data showed that treatment of male mice with TMX at sublethal doses resulted in significant reductions in the levels of EC, Hb, and Plt, as well as an increase in PCV, MCH, and MCHC. Similarly, recent studies indicated that exposure to pesticides covalently bind to DNA due to their highly reactive nature, and they can disrupt metabolic processes by damaging the genetic material [57]. Our results were in agreement with these findings, with TMX inducing significant increases in the rates of DNA damage in hepatic and renal cells. Jameel et al. [58] reported that TMX exposure produced marked declines in the levels of AST and ALT reflect increases in the pathogenesis of multiple kidney and liver injuries has been linked to high levels of LPO caused by ROS. The most prevalent sign of LPO is the production of MDA [52]. The current results clearly demonstrated that this parameter reflective of oxidative stress in the liver and kidneys was significantly increased as a result of a reduction in the overall level of endogenous antioxidants. These findings coincide with those of previous studies [48,53] indicating that TMX administration elevated the level of MDA but decreased the activities of CAT and SOD. Auwal and Kumar [54] revealed that the treatment of rats with TMX induced a substantial increase in renal MDA content. The rise in the level of MDA may be due to the intense formation of ROS targeting cell membranes, consequently reducing the endogenous antioxidant content [55,56].

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Moringa oleifera is widely utilized in alternative medicine for its antioxidant and health-promoting characteristics [60]. Its leaves are a good source of bioactive and phenolic substances, which have been shown to protect against chronic illnesses associated with oxidative damage [61]. Our data showed that MLE (200 mg/kg) did not have any harmful effect on the exposed animals. It attenuated the hematopoietic changes, hepatic and renal dysfunction, MDA formation, and DNA fragmentation induced by TMX. Saleem et al. [62] confirmed the safety of MLE, showing that the acute and sub-chronic administration of MLE to Wistar rats did not appear to result in notable abnormalities in organ relative weights, or hematological and biochemical parameters. The findings of several studies also agreed with our results. For example, Ojo

| Treatments | Tailed cells (μm) | Tail length (μm) | Tail DNA (%) | Olive tail moment (%) |
|------------|------------------|----------------|-------------|----------------------|
| Control    | 7.00 ± 0.58<sup>a</sup> | 7.68 ± 0.31<sup>a</sup> | 11.74 ± 0.37<sup>a</sup> | 0.90 ± 0.06<sup>a</sup> |
| MLE        | 6.33 ± 0.80<sup>b</sup> | 6.67 ± 0.26<sup>b</sup> | 10.66 ± 0.69<sup>b</sup> | 0.72 ± 0.07<sup>b</sup> |
| TMX        | 29.67 ± 1.45<sup>c</sup> | 16.31 ± 0.53<sup>c</sup> | 18.85 ± 0.33<sup>c</sup> | 3.07 ± 0.14<sup>c</sup> |
| TMX + MLE  | 11.33 ± 1.20<sup>d</sup> | 10.47 ± 0.43<sup>d</sup> | 12.67 ± 0.43<sup>d</sup> | 1.33 ± 0.08<sup>d</sup> |

Data are shown as mean ± SE. Different superscripts in the same column represent significant differences (P < 0.05).

The administration of TMX (1/10 LD<sub>100</sub>) to mice caused significant changes in biomarkers associated with liver (AST, ALT, ALP, albumin) and kidney functions (creatinine, urea, uric acid). These findings were in agreement with several studies that revealed the same results [8-10]. It is well established that increases in AST and ALT reflect increases in the permeability of the plasma membrane as a consequence of hepatic injury, so they are considered as common indicators of liver damage [50]. Similarly, increases of creatinine and uric acid concentrations can be attributed to renal impairment, resulting in reduced renal blood flow with a reduction in glomerular filtration rate and a decrease in their excretion [51].

Exposure to pesticides can lead to the intense formation of reactive oxygen species (ROS), which can induce tissue injury by triggering multiple oxidative mechanisms and lipid peroxidation (LPO). The pathogenesis of multiple kidney and liver injuries has been linked to high levels of LPO caused by ROS. The most prevalent sign of LPO is the production of MDA [52]. The current results clearly demonstrated that this parameter reflective of oxidative stress in the liver and kidneys was significantly increased as a result of a reduction in the overall level of endogenous antioxidants. These findings coincide with those of previous studies [48,53] indicating that TMX administration elevated the level of MDA but decreased the activities of CAT and SOD. Auwal and Kumar [54] revealed that the treatment of rats with TMX induced a substantial increase in renal MDA content. The rise in the level of MDA may be due to the intense formation of ROS targeting cell membranes, consequently reducing the endogenous antioxidant content [55,56].

Moringa oleifera is widely utilized in alternative medicine for its antioxidant and health-promoting characteristics [60]. Its leaves are a good source of bioactive and phenolic substances, which have been shown to protect against chronic illnesses associated with oxidative damage [61]. Our data showed that MLE (200 mg/kg) did not have any harmful effect on the exposed animals. It attenuated the hematopoietic changes, hepatic and renal dysfunction, MDA formation, and DNA fragmentation induced by TMX. Saleem et al. [62] confirmed the safety of MLE, showing that the acute and sub-chronic administration of MLE to Wistar rats did not appear to result in notable abnormalities in organ relative weights, or hematological and biochemical parameters. The findings of several studies also agreed with our results. For example, Ojo

![Fig. 2. Photographs of the comet assay in male mouse cells: (a) normal intact cells, (b) cells of the TMX (1/10 LD<sub>100</sub>)-treated group, and (c) those of the TMX + MLE-treated group.](image1)

![Fig. 3. Rates of DNA fragmentation induced by thiamethoxam (TMX) and/or Moringa leaf aqueous extract (MLE) in liver and kidney cells.](image2)
and Adetoyi [63] revealed that MLE achieved substantial improvements in hematopoietic changes compared with the findings in a control. Moreover, Soliman et al. [64] demonstrated that the protective effects of *M. oleifera* against methotrexate toxicity are mediated through the regulation of anti-inflammatory, antioxidant, and antiapoptotic signaling pathways. Chidi et al. [65] also observed that the administration of MLE plus CCl₄ reversed the changes in markers of hepatic dysfunction to near normal ranges. Furthermore, Balogun et al. [66] showed that MLE minimized the impact of gamma radiation exposure associated with oxidative damage. The levels of essential fatty acids in *M. oleifera* could account for its antioxidant ability and ability to stabilize the tubular cell membrane against oxidative damage [67]. It was also shown by Yousef et al. [68] that *M. oleifera* effectively diminished hepatic oxidative damage and markedly improved the content of antioxidants in methotrexate-treated animals. Additionally, Sharifuddin et al. [69] and Alibrahim and Binobead [70] indicated that MLE administration alongside monosodium glutamate for rats reversed the alterations in hepatic function, oxidative damage, and genetic influence through free radical scavenging. Hamed and El Sayed [71] also mentioned that the use of MLE against the pesticide pendimethalin diminished the hepatic dysfunction and also normalized the levels of serum renal biomarkers. Nafiu et al. [72] established that pretreatment with *M. oleifera* extracts prior to gentamycin exposure significantly decreased the concentrations of creatinine, uric acid, and urea, which may have been the result of the therapeutic properties of oleic and linoleic acids [67]. Besides, Farag et al. [73] indicated that treatment with *M. oleifera* greatly reduced the genetic and biochemical destruction induced by aflatoxin B₁ in rats. The ameliorating effects of MLE on genetic materials might be due to its high content of natural antioxidants, which have vital properties as antigentotoxic agents. The mode of action of antioxidants might be due to their binding with the mutagen or suppression of the activation of the cytochrome enzyme system, leading to a reduction of DNA adducts and consequently triggering minimalization of deviations in genetic material [74].

5. Conclusion

In conclusion, this experiment showed that 28 days of exposure to TMX (1/10 LD₅₀) resulted in increased content of MDA, and biochemical and genetic alterations in male mice. *M. oleifera* extract ameliorated these destructive effects of TMX. This study proved the role of the antioxidant function of *M. oleifera* in recovering from the hematological and biochemical changes, antioxidant deficiency, and damage to genetic material induced by TMX exposure to near normal levels.

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Author contributions

AIE and OHE designed the study. OHE and FSA carried out the biochemical and genetic experiments and collected the data. OHE prepared and analyzed the data under the supervision of AIE. OHE co-wrote the first draft of the manuscript. AIE reviewed, edited the manuscript. AIE, OHE and FSA approved the final version of the manuscript for publication.

Conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Aida el makawy reports was provided by there is no. Aida el makawy reports a relationship with there is no that includes:. Fathia Al-Anany reports a relationship with there is no that includes:. Fathia Al-Anany has patent there is no pending to there is no. there is no relationships.

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Declaration of Competing Interest

The authors report no declarations of interest.
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