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The deterrent effect of acetylcysteine against hepatic and renal damage in thiamethoxam exposed rats

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Abstract

The biochemical, oxidative DNA damage, and the histological alterations associated with thiamethoxam (MX) exposure, a second-generation neonicotinoid broadly used in Egyptian agriculture, were assessed. Also, the role of N- acetylcysteine (NAC), (150 mg/kg/ day) on the adverse effect of MX was investigated. Rats were orally preserved with a sub-lethal dose (1/50 LD_{50}) of MX at 31.26 mg/kg/day, five doses/week for 28 days. The MX exposure resulted in a significant decrease in rats' body weight, protein concentration of both serum and urine, sera catalase (CAT), and glutathione peroxidase (GPx) activities compared to control. In addition increase in sera creatinine, urea, bilirubin, alkaline phosphatase (ALP), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were observed. While the assessment of DNA damage revealed significant altitude in 8-hydroxy-2'-deoxyguanosine (8-OH-2DG) levels in both serum and urine samples. The present findings were supported by microscopic observation of liver and kidney tissues. Evidently, thiamethoxam can damage liver and kidney functions impaired the DNA, and cause histoarchitecture lesions in rats at the tested sublethal dose. In addition, NAC supplementation significantly attenuated of MX adverse effect which reflects its protective influence against MX-induced hepatic-nephrotoxicity.

Key words: DNA damage; Hepatotoxicity; Nephrotoxicity; Histopathological analysis; Malondialdehyde

1. Introduction

Thiamethoxam (MX) is a broad-spectrum second-generation neonicotinoid, used as as systemic and contact insecticide to control chewing and sucking pests [1, 2]. MX has applications as foliar/soil and as a seed treatment for use in most agricultural crops, vegetables, cotton and fruits [3, 4]. MX acts as agonists at the nicotinic acetylcholine receptors (nAChRs) subtypes of insects and mammals. It has higher selectivity factors for insects versus mammals [5], with relatively large oral LD_{50} in albino rats (1563 mg/kg bw) indicating low acute mammalian toxicity [6]. Since MX has a long half-life in soils (>350 days), possible risks of occupational and environmental contamination are taken into account [7].

The liver and kidney are playing an important role in the detoxification and removal of numerous xenobiotics and are susceptible to various disorders as a consequence of exposure to environmental contaminants. In a metabolic investigation, the majority of MX (84–95%) was eliminated in the urine with a minor quantity (2.5–6%) in the feces within 24 h, mainly as an intact compound [8]. MX is rapidly and almost completely absorbed following single oral doses in the rats. It is widely distributed in the body and the highest tissue residues are found in the liver, so it is considered to be the central target of MX-induced toxicity since the liver plays the main role in MX metabolism [9]. MX intoxication in rats significantly alters the liver and kidney enzymatic activities suggesting severe hepatic necrosis and kidney injury [10-12].

Many investigations have shown that MX disrupted the balance between reactive oxygen species (ROS) and antioxidant systems, resulting in the progression of oxidative stress. For this reason, the influence of oxidative stress and ROS on MX-associated neurotoxicity, immunotoxicity, hepatotoxicity, nephrotoxicity, genotoxicity, and reproductive cytotoxic effects has been studied [7, 11, 13-16].

Oxidative DNA damage markers; 8-Hydroxy-2-deoxyguanosine (8-OH-2DG) is commonly analyzed for DNA lesions [17]. It is formed as a result of the reaction between DNA and ROS. It founds the link between intracellular ROS increase and genotoxicity, its accumulation affects the DNA replication process by delaying the repair mechanism, whereas misrepaired DNA can result in

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mutations leading to development of chronic diseases [18, 19].

N-acetylcysteine (NAC) is an aminothiol, inexpensive drug, and acts as synthetic precursor of intracellular cysteine and reduced glutathione (GSH). Thus it is considered an important antioxidant as it stimulates GSH synthesis, therefore maintaining intracellular GSH levels and scavenging ROS [20]. Also, NAC improves the viability of human hepatocytes and protects the liver [21] and kidneys [22] against oxidant damage. NAC has an ameliorative effect against many pesticides toxicity such as organophosphate compounds (Yurumez et al., 2007; [23-25], alpha-cypermethrin[26], carbamates [27], and imidacloprid [28]. Accordingly, the present study was aimed to investigate the protective effect of NAC on hepato-nephro- toxicity induced by MX in male rats. Liver and kidney functions enzymes activities, some urine parameters, oxidative stress status, DNA damage in both urine and serum were measured and confirmed by histopathological study of liver and kidney.

2. Materials and Methods

2.1. Insecticide and chemicals

Thiamethoxam (MX) (98%) was supplied from Zhejiang Heben pesticide and chemical Co., Ltd. China. Whilst N-acetylcysteine (600 mg as effervescent instant) was provided by Sedico Pharmaceuticals Co., Egypt. All of the other chemicals were provided by Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Animals

From Alexandria University Faculty of Science animal house, the ninety day-old male albino rat weighing, 144 ± 3 g was obtained, Rats were housed as groups in four plastic cage, five animals / cage, and maintained in laboratory conditions; 25 °C, 12/12-hour light/dark cycle, and humidity of 65–75%. The animals were fed a well-balanced rat diet (60% maize, 20% soybean, 3% mollus, 1.5% brown dust, 0.5% salt, and 0.2% vitamins,) that was purchased from the Agriculture Ministry's Animals Food Manufactory in Embaba, Giza, Egypt, as well as water *ad libitum*, the animals were acclimatized before being examined for two weeks. All animal processes followed the rules of standard protocols, which were confirmed by OECD, (2008) [29].

2.3. Experimental design

Twenty male albino rats were divided into four groups, each with five animals, and orally dosed five times / week for 28 days by gavage as follows: Group (C) was administered to corn oil (0.5 ml/kg bw) and served as a control group. Group (NAC) preserved with 150 mg/kg bw of N-acetylcysteine. Group (MX) exposed to thiamethoxam 31.26 mg/kg bw equivalent to (1/50 $LD_{50;}$ 1563 mg/kg bw cited by Tomlin, (2009) [6]).Group (NAC + MX) concomitantly provided with 150 mg/kg bw of NAC 30 min before dosing the 31.26 mg/kg bw of MX.

2.4. Urine collection

Utilizing metabolic cages animals' urine was collected at the end of dosing for the qualitative examination of pH, blood present, glucose, bilirubin, protein, and 8-OH-2DG.

Urine glucose level was measured by Trinder [30] enzymatic method, the oxidation of glucose to hydrogen peroxide and gluconic acid is catalyzed by glucose oxidase. When hydrogen peroxide is coupled with 4-aminoantipyrine and phenol, a red quinoneimine dye with a maximum absorption of 510 nm is formed. The amount of glucose in the sample is related to the intensity of the red color. Glucose concentration was expressed in mg/dl.

2.5. Blood and organs collection

Animals fasted overnight with free access to water at the end of the treatment period. Then, rats were euthanized using sodium pentobarbital. For serum separation, blood was collected in non-heparinized tubes, centrifuged at 1500 xg for 10 min using a Sigma K30 bench centrifuge, and stored at -20 °C until use. Furthermore, liver and kidneys were swiftly removed, weighted, and preserved in 10% buffered formalin for histological examination.

2.6. Serum biochemical parameters

Functional biomarkers of the liver and kidney were tested in sera using BioMed diagnostic kits (Germany) according to the manufacturer's pamphlet and measured on a PG Instruments Ltd.T-80+UV/VIS spectrometer (Leicestershire, UK).

2.6.1. Liver function biomarkers

2.6.1.1. Assessment of serum alkaline phosphatase (ALP) activity

ALP was determined by the method of Belfield and Goldberg [31]. The method depends on the reaction of phenol, which is released by enzymatic hydrolysis of disodium phenyl phosphate with 4-aminophenazone in the presence of an alkaline oxidizing agent (potassium ferricyanide) to yield a red compound. In brief, 25 µl of serum sample was added to 0.5 ml of substrate buffer (5mM disodium phenyl phosphate dissolved in 50 mM carbonate buffer, pH 10.0). The reaction was incubated at 37 °C for 20 min, and then 250 µl of 50 mM 4-Aminophenazone was added and mixed well. After

that, 250 µl of color reagent (0.2 M potassium ferricyanide) was added and the mixture was allowed to stand for 5 min in the dark at room temperature. Color development was rapid and measured at 510 nm against a reagent blank. The enzyme's activity is given as U/mg protein.

2.6.1.2. Measurement of serum/urine total bilirubin concentrations

Total bilirubin was estimated by the Walters and Gerarde [32] method. Fifty microliters of 90 mM sodium nitrite were added to 1 ml of reagent containing 60 mM sulphanilic acid, 200mM hydrochloric acid, and 10 M dimethylsulphoxide (DMSO). Serum/urine sample (100 µl) was added and reacted with the diazonium salt of sulphanilic acid to produce azobilirubin. In an acid media, DMSO catalyzes azobilirubin production from free bilirubin. The reaction was kept away from light and incubated at room temperature for 10 min. The intensity of the pink color was measured at 535 nm and is proportional to the bilirubin concentration. Total bilirubin concentration was expressed as mg/dl.

2.6.1.3. Measurement of serum/urine total protein concentrations

The Biuret method [33] was used to determine total protein using Albumin as a standard. The method depends upon the formation of a colored complex between protein and cupric ions in an alkaline medium. Briefly, 25 µl of serum sample was added to 1 ml of Biuret reagent (6mM cupric sulfate, 21 mM sodium potassium tartrate, 0.75 M sodium hydroxide, and 6mM potassium iodide) and incubated for 10 min at 37°C. The colour complex's intensity was measured at 550 nm and was shown to be directly related to the protein concentration in the sample. Total protein concentration was expressed in terms of mg/dl.

2.6.2. Kidney function biomarkers

2.6.2.1. Measurement of serum creatinine concentrations

Creatinine was estimated by Schirmeister, et al. [34] which depends on the reaction between creatinine and picrate in an alkaline solution yielding a colored complex. The serum sample was mixed with an equal volume of 1.2 M trichloroacetic acid (TCA) for 5 minutes before centrifugation at 3000 rpm for 10 min. The supernatant (0.5 ml) was added to 0.25 ml of 20 mM picric acid and 0.25 ml of 1.2 mM sodium hydroxide. After incubation for 5 min at 37 °C, the absorbance was measured at 520 nm. The increase in absorbance rate is related to the sample's creatinine concentration (mg/dl).

2.6.2.2. Measurement of serum urea concentrations

Urea was measured by Fawcett and Scott [35] method. Serum sample (10 µl) is decomposed by 200 µl of urease to form ammonia and carbon dioxide. The ammonium ions formed are measured by the Berthelot reaction. After incubation for 5 min at 37 °C, ammonia reacts with 1 ml of 15 mM of sodium hypochlorite and 1 ml of color reagent (containing 100 mM phenol and 0.2 mM sodium nitroprusside) in an alkaline medium. The reaction was incubated at 37 °C for 10 min to form an indophenol. The blue dye indophenol product was measured at 550 nm and was relative to the initial urea concentration (mg/dl).

2.6.3. Oxidative stress biomarkers

SOD, CAT, GPx, and lipid peroxidation (LPO) were measured according to the instructions included in the Biodiagnostic kit, and the principles of several methodologies are listed below for each biochemical parameter.

2.6.3.1. Assessment of serum superoxide dismutase (SOD) activity

SOD was determined using Nishikimi and Rao's technique Nishikimiet al. [36]. The technique is based on the SOD enzyme's ability to prevent nitroblue tetrazolium dye degradation by phenazine methosulphate. Briefly, 0.1 ml sample was mixed with 1.0 ml of 50 mM phosphate buffer pH 8.5, 0.1 ml of 1mM nitroblue tetrazolium (NBT) and 0.1 ml of 1mM nicotinamide adenine dinucleotide dehydrogenase (NADH). The reaction was started by adding 0.1 ml of 0.1 mM phenazine methosulphate (PMS), and then absorbance was taken at 560 nm for 5 min. SOD activity was recorded as U/mg protein.

2.6.3.2. Assessment of serum Catalase (CAT) activity

CAT level was assayed spectrophotometrically according to the method of Aebi [37]. The assay was performed with 50 µl of sample in 0.5 ml of 100 mM phosphate buffer, pH 7.0. The reaction was started by adding 0.1 ml of 0.5 mM H_2O_2 and incubated at 25°C for 1 min, where CAT reacts with a known quantity of H_2O_2. The reaction was then stopped by adding 0.2 ml of catalase inhibitor. The amount of H_2O_2 remaining in the reaction mixture is then determined by the oxidative coupling reaction of 0.5 ml of 2 mM 4-aminoantipyrene and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) in the presence of horseradish peroxidase (HRP). The mixture was incubated for 10 min at 37°C. The resulting quinoneimine dye is measured at 510 nm and the intensity of the color is inversely associated with the amount of catalase in the original sample. The activity of CAT was expressed as U/mg protein.

2.6.3.3. Assessment of serum glutathione peroxidase (GPx) activity

The GPx activity was estimated based on the oxidation of GSH and reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) using glutathione reductase Paglia and Valentine [38]. The

reaction mixture contained 1 ml of 50 mM phosphate buffer with 0.1 % Triton X-100 (pH 7.0), 0.1 ml of NADPH reagent (4.8 mM GSH, 0.96 mM NADPH and 12 units glutathione reductase) and 0.01 ml of sample. The reaction was initiated by adding 0.1 ml of H_2O_2 as substrate. GPx activity was measured as the reduction in absorbance at 340 nm for 3 min. The activity of the enzyme was expressed as mU/mg protein.

2.6.3.4. Measurement of serum lipid peroxidase (LPO) level

According to a colorimetric method of Kei [39] Lipid peroxidation was measured using thiobarbituric acid reactive substances (TBARS) and represented in terms of malondialdehyde (MDA) concentration. A serum sample of 200 µl was mixed well with 1 ml of 25 mM thiobarbituric acid (TBA) solution, boiled for 30 min and then cooled. The absorbance was recorded at 534 nm. The concentration of MDA was expressed as nmol/mg protein

2.6.4. DNA damage assay

The 8-hydroxy-2′-deoxyguanosine (8-OH-2DG) content in serum and urine as a marker to DNA damage was measured by Sandwich-ELISA (enzyme-linked immunosorbent assay) method. Analyzes were performed using Quick Detect 8-OH-2DG (Rat) ELISA Kit, BioVision, USA (Catalog # E4442-100). According to the test procedure, 50 µl of standard (1.5, 3, 6, 12, 24, 48 ng/ml) or diluted sample was added to a pre-coated plate with 8-OH-2DG. Then, with the exception of the blank control well, 100µl of HRP-Conjugate reagent was applied to each well. At 37°C, the microplate was covered and incubated for an hour. The wells were washed 5 times with a wash solution. To each well 50 µl of chromogen solution A and 50 µl of chromogen, solution B was added and incubated at 37°C for 15 min in the dark. Stop solution of 50 µl was added to each well to terminate the reaction and change the blue color to yellow. Finally, the absorbance of the standard and samples was recorded at 450 nm by a microplate reader (STAT FAX-2100, Awareness Technology, Inc., Palm City, Florida, USA). The calibration curve was plotted for the absorbance values obtained and the 8-OH-2DG levels were determined as ng/ml against the absorbance of the samples. The original concentration was calculated by multiplying the dilution factor (5).

2.7. Histopathological analysis

Carleton's histology approach was used to do the histological study [40]. Liver and kidneys tissues of control and thiamethoxam dosed rats were preserved in 10% buffered formalin, dried and embedded in paraffin using standard techniques. Light microscopy was used to examine the hematoxylin and eosin (H&E) stained sections with 5 µm thick.

2.8. Statistical analysis

Data have been presented as mean ± standard error (SE). The statistical significance standard was set at $p \leq 0.01$. For data analysis, one-way variance analysis (ANOVA) was employed, followed by the Student-Newman-Keuls Test using version 25.0 of IBM SPSS software (Statistical Package for Social Sciences, Chicago, IL, USA Ibm) [41] .

Results

3.1. Changes in Body, organ, and relative organ weights

Results in Table 1 showed that there was no mortality after repeated oral administration of thiamethoxam (MX) at 31.26 mg/kg bw day for 28 days (5 doses/week), however, some signs of toxicity such as diarrhea, glossy eye, and drooling were observed at the end of the exposure period. At the present data, the animals given MX showed a substantial ($p \leq 0.01$) decrease in the percentage of body weight changes by 46.59% and a significant ($p \leq 0.01$) rise in the relative weights of the liver by 15.65% while the kidney relative weights was statistically ($p \leq 0.01$) reduced by 2.4% compared to the control group (Table 1). While the rats treated with N-acetylcysteine (NAC) alone showed significant improvement in final body weight calculated by 2.3%. The group treated with MX only showed a significant reduction by 11.5% meanwhile the concomitant treatment of NAC plus MX significantly ($p \leq 0.01$) modulated the reduction in the final body weight to 6.85% compared to control weights (Table 1).

3.2. Urine biochemical analysis data

Measurements of urine pH, presence of blood, glucose, protein, and bilirubin levels were summarized in Table 2. Statistical ($p \leq 0.01$) increases of urine pH by 1.25 fold in rats exposed to MX while NAC concomitant with MX attenuated the MX alkaline effects to become 1.11 fold compared to the control group. Thiamethoxam-treated animals exhibited a significant reduction in urine protein concentration by 49.2% while the co- administration of NAC with MX modulated the protein level to be 11.47% compared to control animals. No blood cells, glucose, or bilirubin were found in urine samples of MX treated rats equaled to the control group (Table 2).

3.3. Sera biochemical analysis data

The obtained results in Table 3 were exhibited an increase in sera creatinine, urea and, bilirubin levels by 3.1, 1.75, and 2.65 folds, respectively compared to control rats. Whereas the

concomitant treatment of NAC with MX mitigated the MX effect to 0.93, 1.1, and 0.88 folds for creatinine, urea, and bilirubin, respectively related to control values. Significant ($p \leq 0.01$) decreases in serum total protein by 58.4% in MX treated rats' group while NAC plus MX treated group showed improvement in the previous ratio of serum total protein to be 33.8% lower than control one. MX exposed animals showed a greatly ($p \leq 0.01$) rise in ALP activity by 396.5% while the treatment of NAC concomitantly with MX alleviated the MX aggressively effect to 48.26% compared to the control group.

3.4. Changes in the sera antioxidant and oxidative stress levels

Table 4 data showed significant increased both the SOD and MDA levels by 33.29% and 454.02%, respectively while the dosing of NAC concomitantly with MX mitigated MX harmful effect towards SOD and MDA to be 29.3 and 124.15%, respectively corresponding to control group. Contrarily, CAT and GPx significantly ($p \leq 0.01$) exhibited low activities in MX exposed animals represented by 52.0% and 33.6%, respectively while the animal group treated with NAC plus MX advanced the MX effect to become 24.14% and 9.1% for CAT and GPx, respectively compared to control levels.

3.5. Changes in serum and urine 8-hydroxy-2'-deoxyguanosine (8-OH-2DG) levels

Monitoring the DNA damage in serum and urine samples of male albino rats orally treated with 1/50 LD_{50} of thiamethoxam was illustrated in Table 5. Rats exposed to MX showed significant ($p \leq 0.01$) elevation in 8-OH-2DG levels in both serum and urine samples by 428.9% and 234.6%, respectively. While the animal group supplied NAC with MX treatment greatly ($p \leq 0.01$) attenuated the DNA damaging effect of MX to be 49.1% and 50.18% for serum and urine, respectively compared to the control group.

3.6. Histopathological analysis

The light microscopic investigations of the livers in Figure (1) illustrated that liver of control (A) and NAC (B) groups showed normal architecture and pathological-free hepatic central vein (CV), portal triad (Pt), patent sinusoids (S), regular and normal arrangement of the hepatocytes (H). The MX treated rats (C & D) showed symptoms of degenerative changes in the hepatocytes such as; hemorrhage (Hm), severe congestion (SG) of the hepatic central vein (CV), destructive cholangitis (Dg), hepatic inflammation (HF), necrosis (N), hepatic degeneration (Hd), congestion (G) within CV, Fibrosis (F), and inflammatory cellular infiltration (Fi). The portal triad and central vein showed nominal histological changes and focus inflammation (Fo) within hepatocytes (H) suggesting recovery after co-administration of NAC, indicating that NAC relieved the damages induced by MX dosing in rats (Fig. 1, E).

Figure (2) represents kidney sections from the control group (A) and NAC-treated rat (B) showed intact histological structure of glomeruli and renal tubules. However, abnormalities in the kidney of MX treated rats were detected in glomeruli and in convoluted tubules. The main characteristic findings were the appearance of; congested degenerative glomerular tuft, swelling of the tubule lining epithelium, and inflammatory cell infiltration

Table 1 Changes in body and organs weight of male albino rats orally administrated with thiamethoxam (31.26 mg/kg bw), N- acetylcysteine (150 mg/kg bw) and their combination for a month (5 doses/week).

Animal groups / weights	Control	N- acetylcysteine	Thiamethoxam	N- acetylcysteine + Thiamethoxam
Initial body weight	$145^{a} \pm 0.71$	$142.8^{b} \pm 0.48$	$142.8^{b} \pm 0.25$	$142.2^{b} \pm 0.26$
Final body weight	$185.3^{a} \pm 0.33$	$189.6^{a} \pm 1.44$	$164.0^{c} \pm 2.08$	$172.6^{b} \pm 2.18$
Body weight changes (%)	$27.79^{b} \pm 0.12$	$32.77^{a} \pm 0.23$	$14.84^{d} \pm 0.11$	$21.37^{c} \pm 0.09$
Liver weight	$5.80^{a} \pm 0.28$	$6.04^{a} \pm 0.46$	$5.93^{a} \pm 0.57$	$5.94^{a} \pm 0.52$
Relative liver weight	$3.13^{a} \pm 0.022$	$3.18^{a} \pm 0.012$	$3.62^{c} \pm 0.015$	$3.44^{b} \pm 0.023$
Kidney weight	$1.21^{ab} \pm 0.026$	$1.26^{a} \pm 0.086$	$1.18^{b} \pm 0.056$	$1.22^{ab} \pm 0.082$
Relative kidney weight	$0.65^{c} \pm 0.002$	$0.66^{c} \pm 0.023$	$0.72^{a} \pm 0.031$	$0.70^{b} \pm 0.024$

Values denote the mean ± SE of five animals/group. a, b, c, & d different superscripts indicating different statistical significant differences between groups using one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test at $p \leq 0.01$. Body weight changes (%) = (Final weight of the body - Initial weight of the body / Initial weight of the body) x 100
Relative weight of the organ = (Organ weight / Final weight of the body) x 100

Table 2 Urine analysis data of male albino rats orally administrated with thiamethoxam (31.26 mg/kg bw), N-acetylcysteine (150 mg/kg bw) and their combination for a month (5 doses/week)

Animal group	Blood (RBC/µl)	Glucose mg/dl	protein mg/dl	Bilirubin mg/dl	pH
Control	nill	nill	$6.1^{a} \pm 0.033$	nill	$7.1^{d} \pm 0.057$
N-acetylcysteine	nill	nill	$6.0^{a} \pm 0.057$	nill	$7.4^{c} \pm 0.012$
Thiamethoxam	nill	nill	$3.1^{c} \pm 0.032$	nill	$8.9^{a} \pm 0.028$
N-acetylcysteine + Thiamethoxam	nill	nill	$5.4^{b} \pm 0.034$	nill	$7.9^{b} \pm 0.029$

Urine analysis by a group, each values denote the mean ± SE of ten animals/group. a, b, c, & d different superscripts indicating different statistical significant differences between groups using one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test at $p \leq 0.01$.

Table 3Sera analysis data of male albino rats orally administrated with thiamethoxam (31.26 mg/kg bw), N-acetylcysteine (150 mg/kg bw) and their combination for a month (5 doses/week)

Animal group	Createnine mg/dl	Urea mg/dl	Bilirubin mg/dl	Total protein mg/dl	ALP (U/mg protein)
Control	$0.55^{b} \pm 0.005$	$24.56^{c} \pm 0.033$	$0.49^{b} \pm 0.002$	$7.60^{a} \pm 0.40$	$2.30^{c} \pm 0.026$
N- acetylcysteine	$0.50^{c} \pm 0.004$	$24.53^{c} \pm 0.032$	$0.52^{b} \pm 0.005$	$7.65^{a} \pm 0.033$	$2.39^{c} \pm 0.026$
Thiamethoxam	$1.69^{a} \pm 0.003$	$42.93^{a} \pm 0.087$	$1.30^{a} \pm 0.008$	$3.16^{d} \pm 0.033$	$11.42^{a} \pm 0.28$
N-acetylcysteine + Thiamethoxam	$0.51^{c} \pm 0.001$	$27.00^{b} \pm 0.057$	$0.43^{c} \pm 0.008$	$5.03^{c} \pm 0.023$	$3.41^{b} \pm 0.044$

Values denote the mean ± SE of five animals/group. a, b, c, & d different superscripts indicating different statistical significant differences between groups using one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test at $p \leq 0.01$

Table 4 Changes in the sera antioxidant and oxidative stress levels of male rats orally administrated by thiamethoxam (31.26 mg/kg bw), N-acetylcysteine (150 mg/kg bw) and their combination for a month (5 doses/week).

Animal groups	SOD (U/mg protein)	CAT (U/mg protein)	GPx (mU/mg protein)	MDA (nmole/mg protein)
Control	$162.46^{b} \pm 0.49$	$385.33^{b} \pm 0.86$	$96.10^{b} \pm 0.110$	$199.2^{d} \pm 0.42$
N-acetylcysteine	$171.16^{a} \pm 0.64$	$462.50^{a} \pm 1.32$	$109.42^{a} \pm 0.290$	$253.3^{c} \pm 1.21$
Thiamethoxam	$108.38^{d} \pm 0.32$	$184.94^{d} \pm 0.34$	$63.80^{d} \pm 0.150$	$1103.62^{a} \pm 3.01$

N-acetylcysteine + Thiamethoxam	$114.8^{c} \pm 0.11$	$292.30^{c} \pm 0.35$	$87.40^{c} \pm 0.358$	$446.51^{b} \pm 2.35$

Values denote the mean ± SE of five animals/group. a, b, c, & d different superscripts indicating different statistical significant differences between groups using one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test at $p \leq 0.01$.

Table 5 Oxidative DNA damage in serum and urine of male albino rats orally administrated with thiamethoxam (31.26 mg/kg bw), N- acetylcysteine (150 mg/kg bw) and their combination for a month (5 doses/week)

Animal group	8-OH-2DG (nmole/mg protein)	
	Sera	Urine
Control	$21.59^{c} \pm 0.41$	$18.83^{c} \pm 0.035$
N-acetylcysteine	$21.90^{c} \pm 0.63$	$16.85^{d} \pm 0.034$
Thiamethoxam	$114.19^{a} \pm 1.95$	$63.01^{a} \pm 0.18$
N-acetylcysteine + Thiamethoxam	$32.18^{b} \pm 0.48$	$28.28^{b} \pm 0.53$

Values denote the mean ± SE of five animals/group. a, b, c, & d different superscripts indicating different statistical significant differences between groups using one-way analysis of variance (ANOVA) and the Student-Newman-Keuls test at $p \leq 0.01$.

between the deteriorated tubules with fibrosis and hyalinosis between the tubules in a focal manner, disturbing proximal tubules, hyperchromatic mesangial cells, and swelling Bowman's capsule were also observed. Co-administration of the NAC with MX showed marked improvement in kidney histological structure compared to the MX group alone (Fig. 2, E).

4. Discussion

Body, organ, and relative organ weights are essential factors for toxicity evaluation in toxicological research [42]. The present results showed a decrease in body weight, and this may be attributed to reduced food consumption as a result of diarrhea and food avoidance after the repeated exposure of MX which was observed at the end of the exposure period. In accordance with the present data El Okle, et al. **[**13**]** revealed a significant decline in body absolute weight in rabbits treated with 250 mg/kg b.w of MX for 90 successive days. Also, Abouelghar, et al. [15] concluded that administration to 30 mg/kg b.w./day of MX for 28 days decreased the final mice body. In line Mansour and Mossa [43] postulated that the loss in body weight could be associated with a reduction in food intake (anorexia or food avoidance), poor food digestibility, or elevated lipid and protein breakdown due to treatment-related toxicity. Similar results were obtained by El-Masry, et al [44] following the exposure of animals to Lambda-cyhalothrin and different pyrethroid compounds [45]. Changes in an organ's weight either absolute or relative after administration of a xenobiotic have been reported to reflect the chemical's harmful effect [46]. Present findings revealed an increase in relative liver weight in MX treated rats which could be related to MX toxic effect, and those are consistent with Abouelghar, et al. [15] who recorded an increase in the relative weights of liver and kidney in MX-treated rabbits. Furthermore, Reda [47] reported an increase in relative liver weight after exposure to 2.6 mg/kg bw of imidacloprid in mice. Changes in kidney weight, according to Sellers, et al. [48], could indicate renal toxicity, hypertrophy of renal tubular, or chronic progressive nephropathy.

Urine is a useful fluid for demonstrating a variety of biochemical and physiological concepts. The present experiments revealed an increase in urine pH, which could indicate that the body is attempting to eliminate MX, as MX stability is known to diminish with increasing alkaline environments [49, 50]. This study

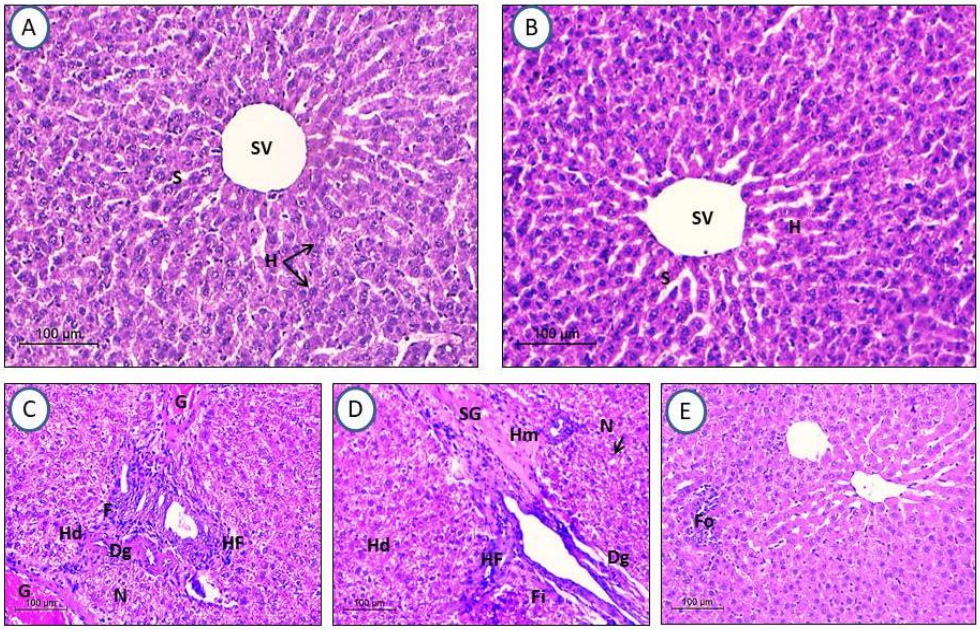

Figure 1. Microscopic image of the liver tissues of rats treated with thiamethoxam (1/50 of LD_{50}; 31.26 mg/kg bw) (C & D), N- acetylcysteine (150 mg/kg bw) (B), and their combination (E) for a month (5 doses/week). Control group dosed corn oil (A), (C&D) displays a destructive cholangitis (Dg), hemorrhage (Hm), severe congestion (SG) of the hepatic central vein (CV), hepatic inflammation (HF), necrosis (N), hepatic degeneration (Hd), congestion (G) within the CV, Fibrosis (F), inflammatory cellular infiltration (Fi), and (E) focus inflammation (Fo) in hepatocyte (H). (H & E 200X)

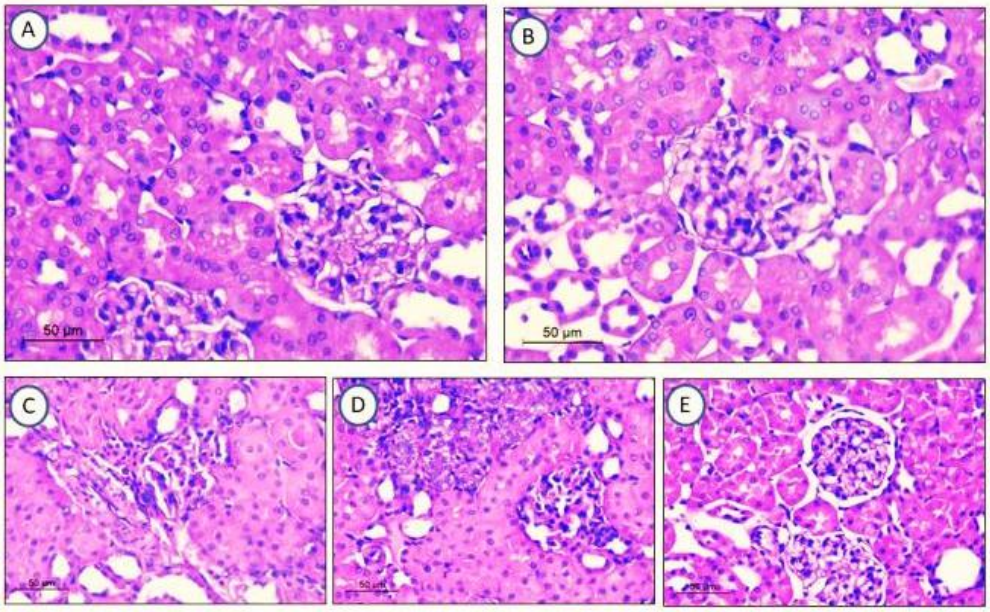

Figure 2. Microscopic image of the kidney tissues of rats treated with thiamethoxam (1/50 of LD_{50}; 31.26 mg/kg bw) (C & D), N- acetylcysteine (150 mg/kg bw) (B), and their combination (E) for a month (5 doses/week). Control group dosed corn oil (A), (A & B) Bowman's capsule, proximal tubules, and urine space are all in pathological free, (C & D) Showing congested degenerative glomerular tuft, swelling of the tubule lining epithelium, and inflammatory cell infiltration between the deteriorated tubules with fibrosis and hyalinosis between the tubules in a focal manner, swelling and lose architecture Bowman's capsule, and (E) minor alteration in capsule shapes, mild focal inflammation, and normal arrangement of tubules in the cortical part signifying recovery. (H & E 400X).

determined that there are no blood cells, glucose, or bilirubin in the urine that could affect urine capacitance measurements as a result of MX treatment, which is consistent with the findings of Arfat, et al. [51], who indicated the negative presence of blood cells, glucose, or bilirubin in the urine of mice given 5, 10, and 15 mg/kg bw imidacloprid for 15 days. Previous results of Nurulain, et al. [52] revealed that urinalysis indicated a normal glucose level, a significant increase in blood cells number, and a non-significant increase in protein and bilirubin levels in sub-chronic exposure of paraoxon injected rats compared with the control. Another study showed the increase in glucose levels may be a result of chronic exposure to pesticides [53]. In addition, Martin-Reina, et al. [54] found a substantial increase in glucose levels in women farmers who harvest fruits and vegetables when compared to a non-occupational group of women exposed to pesticides but living in the same rural setting.

Urea and creatinine are nitrogenous end products of metabolism and measurements of their levels both reflect the glomerular filtration rate (GFR), which is the parameter that characterizes kidney function. After MX treatment, rats' serum creatinine and urea levels increased considerably, indicating purine and pyrimidine breakdown as well as decreased renal function. A rise in creatinine and urea in the serum of rats subjected to hazardous chemicals, such as pesticides, have been reported in several studies [55-58]. In line with the present results, Yassin, et al. [59] recorded a general increase in serum urea, creatinine, and bilirubin of chlorpyrifos intoxicated rabbits while a diminished total protein level was also observed . In agreement with Bharti and Rasool [60] who recorded the presence of higher amounts of urea and creatinine in the serum of malathion-exposed fish after 12 days, indicating renal dysfunction. Correlation between pesticides and total bilirubin levels has been reported by various researchers. An observational study [61] noticed that continuous and prolonged exposure to organophosphorus pesticides elevated bilirubin and liver enzymes levels. SNH [62] revealed that almost all the farmworkers from different stations, in the Gadap area, Pakistan, showed elevated levels of bilirubin. Likewise, Fahimul-Haq, et al. [63] concluded that all pesticide factory workers had normal total and direct bilirubin levels but on the higher side as compared to control volunteers. Pediatric research by Shibazaki, et al. [64] revealed that pesticide exposure during pregnancy may promote oxidative hemolysis in the mother, resulting in newborn hyperbilirubinemia. Among biochemical parameters, protein profiles could be used to detect chronic effects of multiple occupational pesticide exposure [65]. They estimated the effects of pesticide spraying on serum proteins in Indian pesticide sprayers and found that serum total protein and serum albumin were significantly decreased. Contrarily, Serum total protein, albumin, and globulin were significantly increased in Gaza Strip farm workers [66]. In addition, Sankhala, et al. [67] discovered a dose-dependent reduction in serum total protein and globulin in acephate-treated mice.

In people and experimental animals, increased serum alkaline phosphatase (ALP) activity is a sign of hepatobiliary injury. Following the present work, a rise in serum ALP activity was documented in a study investigating the hazardous effects of fenvalerate, isoproturon, ziram, and their mixture given orally to rats [68]. In addition, they found that the mixture of three insecticides interacts antagonistically with the alkaline phosphatase enzyme [68]. Also, Mossa, et al. [69] mentioned that sera ALP activity in male rats were significantly increased as a result of sub-chronic exposure to the fipronil for 45 days. El Okle, et al. [13] founded that MX-treated rabbits (250 mg/kg b.w for 90 successive days) had hepatotoxicity confirmed by attenuation of liver enzyme activities (AST, ALT, and ALP). In line Abdo, et al. [70] recognized an elevation in the activity of ALP in the malathion inhaled rats. Mongi, et al. [71] found statistical increases of serum ALP in rats exposed to 63 mg/kg b.w of chlorpyrifos for 2 weeks. In agreement with Feki, et al. [12] recognized a significant ($P \leq 0.05$) increase in the activity of AST,ALT, ALP, and LDH, this is coordinated with a decrease in the levels of total protein and albumin in the MX treated rats.

Enzymatic antioxidants are endogenous antioxidant systems with primary and secondary defensive actions. The principal defences that prevent or neutralize reactive species are SOD, CAT, and GPx [72]. SOD can protect cells from oxidative stress induced by free radicals via catalysing the conversion of superoxide anion free radicals to O_2, which is subsequently converted to H_2O_2 by CAT or GPx. The current study showed that there is a significant decline in the activities of SOD, CAT, and GPx, along with a tremendous increase in MDA level in MX treated group. These results could be attributed to many factors. Firstly, MX cause oxidative stress by forming free radicals, implying that the mitochondrial respiratory chain is dysfunctional [73], and secondly, they disrupt antioxidant homeostasis, leading to antioxidant depletion [74]; as well as disrupt redox processes, thereby altering

antioxidant enzyme activities and increasing lipid peroxidation [75]. Also, Yan, et al. [16] concluded that MX induced oxidative stress in Zebra fish livers which confirmed by the increased SOD, CAT, and GPx activities. A study of oxidative stress among 48 agricultural workers exposed to OPs [76] demonstrated that the exposed group's blood SOD levels were considerably lower than the non-exposed group's. According to, Hernández et al., oxidative stress caused by pesticide exposure might cause SOD activity to be inhibited, resulting in superoxide radical buildup [77]. Interaction of reactive oxygen species with the thiol group present at the catalytic center of the enzyme is a possible mechanism for pesticides inhibiting SOD activity, and previous studies have shown that superoxide radicals can inhibit CAT and GPx activities, resulting in excessive hydrogen peroxide production and SOD inhibition [78]. Other investigations in Thailand and Egypt showed the same decrease in the SOD activity in the exposed groups [79, 80]. In contrast to these findings, several researchers, such as Simonian, et al. [81] observed an increase in SOD levels in the exposed group. Also, Vidyasagar, et al. [82] showed that RBC and plasma SOD was increased in patients with organophosphorus insecticide poisoning vs. the control group. In addition, Ahmadi, et al. [83] observed a 61.0 percent rise in SOD, and they attributed it to the activation of the compensatory mechanism by pesticide-induced overproduction of reactive species [77].

CAT is a cellular defense system that dismutases H_2O_2 into H_2O and O_2. Although it is primarily produced in the liver, kidney, and erythrocytes, CAT has been detected in almost all organs. Our findings are consistent with the findings of many other researchers who found that the CAT activity was decreased when rats were treated with 6 mg/kg bw deltamethrin for seven days [84], dimethoate [85], diazinon [86], chlorpyrifos, methyl parathion, and malathion alone or in combination [87]. GPx transforms glutathione (GSH) to its oxidized form (GSSG), H_2O_2 to H_2O, and lipid hydroperoxides (ROOH) to the stable alcohols. This enzyme protects cell membranes, red blood cells, and haemoglobin from oxidative damage. In line with the present data Wasef, et al. [88] found that fipronil-treated rats showed a substantial reduction in hepatic GPx activity. Also, Mossa, et al. [86] posted that administration of acetylsalicylic acid, diazinon, and their combination leads to significant decreases of GPx activity in rats' erythrocytes. In rat tissues, Ojha, et al. [87] stated that exposure to chlorpyrifos, methyl parathion, and malathion alone or in combination caused dose-dependent decreases in GPx activity.

Increased MDA content is vital evidence of lipid peroxidation (LPO) [89]. MDA is a primary oxidation product of peroxidized polyunsaturated fatty acids. It has been proposed as one of the molecular pathways implicated in the toxicity of xenobiotics (e.g. pesticides) [90]. In line with our obtained results A Hussein, et al. [91] and Abd-Elhakim, et al. [92] observed an increase in the levels of MDA in imidacloprid intoxicated rats. Also, mice intoxicated with 30 mg/kg b.w./day of thiamethoxam for 28 days showed increase in the oxidative stress parameters such as MDA level in association with decrease in total antioxidants level [15]. A study of rats treated with 6 mg/kg bw deltamethrin for seven days [84] observed a significant increase in serum MDA. Several reports recorded increased MDA hepatic levels in fipronil and dimethoate treated rats [85, 88]. In addition**,** Elshamy, et al. [76] reported a higher blood MDA level among agricultural workers compared to the non-exposed group following OPs exposure.

The suppression of antioxidant enzymes (SOD, CAT, and GPx) reported in this study implies that the antioxidant defense system is unable to cope with the input of free radicals caused by MX exposure. Suppression of enzymes involved in free radical eradication causes H_2O_2 to clump together, promoting LPO and DNA amendment, as well as altered gene expression [93]. The crosslinking of their molecules produced by the production of MDA-protein adducts, which alters their modulation, could explain the decrease in the aforementioned enzymatic activity.

8-OH-2DG, a repair product of oxidized guanine lesions, has been related to increased oxidative stress or disease states and can be employed as a reliable biomarker for oxidative DNA and RNA damage and repair [94, 95]. According to Zhang, et al. [96], there is a linear relationship between ROS production and the generation of 8-OH-2DG, showing that ROS can initiate the development of 8-OH-2DG. Pesticides may play a significant role in promoting oxidative stress, according to evidence on the increased risk of elevated 8-OH-2DG among farmers exposed to various pesticides [97]. The current results revealed an enormous elevation in 8-OH 2DG levels in both serum and urine samples. In agreement with Lonare, Kumar [98], who observed that exposure of rats to imidacloprid induced oxidative stress and DNA damage in their reproductive organs. In a sample of 31 persons, Muniz, et al. discovered the

concentrations of urine 8-OH-2DG were greater by 2.3 and 8.5 times in sprayers and agricultural workers respectively. Furthermore, high levels of pesticide exposure were connected to an increased risk of genotoxic damage among Bolivian farmers [99]. In a previous study, increased levels of urine 8-OH-2DG were connected to increased exposure to organophosphorus insecticide [100]. Pesticide sprayers who had been exposed to organophosphate pesticides for a long period also showed greater levels of 8-OH-2DG [101]. Otherwise, Tope and Panemangalore [18] discovered no differences in urine 8-OH-2DG levels between pesticide sprayers but detected higher levels in their plasma 8-OH-2DG samples when compared to the control. Also, A Hussein, et al. [91] showed a significant increase in DNA damage detected by comet assay in imidacloprid intoxicated rats. Another study of MX- exposed zebrafish showed induction of oxidative stress and DNA damage which can be detected by the comet assay. [16] An evident signs of genotoxicity in MX-exposed rats, were determined by total changes in hematological parameters and a significant chromatin degradation was seen in the micronucleus test, also complete DNA breakage and damage which assessed by nicking assay [12]

N- acetylcysteine (NAC) is a synthetic precursor of intracellular cysteine and GSH, and it has an anti-ROS effect as scavenges free radicals, either directly through the redox potential of thiols or indirectly by raising GSH levels in cells [20, 102]. The current findings clearly show that NAC can protect the liver and kidneys of rats from oxidative stress and DNA damage caused by MX intoxication. This was achieved by lowering serum creatinine, urea, bilirubin, and ALP activity while raising total protein levels, SOD, CAT, and GPx activities, and decreasing MDA levels, as well as reducing DNA damage by lowering urine and serum genotoxic lesion, 8-OH-2DG. The present data is in parallel with many previous studies stated that NAC treatment has antioxidant, anti-inflammatory, and anti-apoptotic effects against chlorpyrifos-induced neurotoxicity in rat brain tissue [103]. In addition, Osman, et al. [25] concluded that supplementing NAC with chlorpyrifos-ethyl and chlorpyrifos-methyl recovered the tested biochemical and genetic biomarkers in the exposed rat Aboubakr, et al. [23] investigated the therapeutic benefits of N-acetylcysteine and recommended that it be used to treat malathion-induced biochemical, molecular, and histopathological changes. To mitigate the toxicity of certain OPs, NAC can be utilized as a preventive and therapeutic drug [24]. NAC, in combination with sodium bicarbonate [104] or atropine Shadnia [105] was also advised for treating humans to alleviate the negative effects of OPs, such as shortening hospital stays. In line with Abouelella, et al. [106] who cited that, NAC reduces nicotine-induced oxidative stress and reproductive damage in male rats.

Pesticide effects in different animal tissues and organs can be noticed quickly using histological changes [107]. The current histological analysis of treated rats revealed that thiamethoxam intoxication harmed the hepatic tissues' structure. The liver is an important organ in metabolism, and it is the first organ to be exposed to poisons due to its portal blood supply [108]. Destructive cholangitis is a disorder in which the bile duct epithelium destroys followed by inflammation and eventually portal fibrosis in the hepatic lobules, was a typical histological sign of MX toxicity. Inline, degeneration, coagulative necrosis, and hemorrhaging were seen in the chicken liver section after exposure to chlorpyrifos [109]. After four weeks of chlorpyrifos exposure, rat liver showed congestion of the central vein, diffuse Kupffer cell growth, mononuclear cell infiltration, pyknosis, and eosinophilic cytoplasm. [110]. Likewise, Acker, et al. [111] detected oxidative damage in the rat liver tissues. Another notable symptom of MX poisoning was liver hemorrhage, which increased the pressure within the portal vein, leading to portal hypertension. It is frequently due to liver cirrhosis. The present data are in accordance with Mamun, et al. [112] who noted a rise in the sinusoidal space, hepatocyte vacuole formations, and blood vessel congestion with hemorrhage in the hepatic tissues of cypermethrin intoxicated mice. A smaller dose of cypermethrin caused minor hepatic lamina disorganization, while a greater dose caused hepatic cells necrosis with pyknotic nuclei and sinusoidal dilatation with extensively damaged hepatic lamina [113]. Mohany, et al. [114] cited that after 4 weeks of therapy with 0.21 mg/kg imidacloprid, the rat liver had congested central vein and blood sinusoids, widely dispersed pyknotic nuclei, and leukocyte infiltration.. Moreover, Arfat, et al. [51] observed histological alterations such as central vein and sinusoids dilatation, liver, and renal toxicities in imidacloprid treated rats.

The present findings of rat kidney tissues treated with MX revealed many histological changes characterized by swelling in Bowman’s capsule and that may be related to MX causing glomerular hyperfiltration, a state of overwork in the glomeruli, similar toxicity signs for other pesticides were also previously reported by El Okle, et al. [11] and Khaldoun-Oularbi, et al. [14]

who recognized that the renal histoarchitecture of the MX intoxicated rats showed swelling in the glomerular tuft endothelium, swelling in the tubule lining epithelium, and inflammatory cells infiltrate in between the deteriorated tubules. Similarly, kidney tissues of MX-treated rats showed histological alterations [15] . In addition, mice given a mixture of cypermethrin and endosulfan orally showed medullary congestion in their kidneys [115].

The co-administration of the N-acetylcysteine **(**NAC) with MX showed marked improvement in kidney histological structure which is in agreement with Kheradmandi, et al. [116] who demonstrated that intraperitoneal injection of NAC, in combination with CPF ameliorates the pesticide's adverse effects on mice testis. In addition, Osman, et al. [25] supposed that NAC can be utilize to attenuate the toxicity of chlropyrifos-ethyl and chlropyrifos-methyl. On a histological level, the addition of NAC to the hepatocyte isolation process could increase the availability of hepatocytes for transplantation [21]. NAC has also been shown to ameliorate SOD depletion caused by the anti-cancer medication ifosfamide, as well as raise lower Ca^{+2} levels, and protect renal tubules and glomeruli from morphological damage [117].

5. Conclusion

The study demonstrated that thiamethoxam induced hepatic nephrotoxicity in male albino rats. The treatment by thiamethoxam under the tested dose (1/50 LD_{50}) stimulated the MDA as an LPO product and 8-OH-2- DG (a DNA damage biomarker) in both sera and urine samples. Also, kidney and liver functions (creatinine, urea, bilirubin, and ALP) were enhanced in sera. While data analysis revealed a negative response concerning the presence of blood, glucose, or bilirubin in the urine sample. In addition, the treatments caused a significant reduction in SOD, CAT, and GPx in the rat sera. The change of these parameters proposes the involvement of free radical intermediate in the toxicity of thiamethoxam. The toxicological effect of the tested insecticide was confirmed by histological analysis of the liver and kidney. The present study showed that co-administration of NAC protects the liver and kidneys from thiamethoxam-induced oxidative stress, DNA damage, excessive lipid peroxidation, and maintains enzymatic antioxidants near-normal levels. This requires preventative measures to counteract the adverse effects of thiamethoxam.

Conflicts of interest
There are no conflicts to declare.

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