CYMBOPOGON CITRATUS ALLEVIATES MANCOZEB- MEDIATED RENAL TOXICITY IN A RAT MODEL THROUGH COMBATING INFLAMMATION, OXIDATIVE STRESS, AND APOPTOSIS

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ABSTRACT

Background: One of the most widely used dithiocarbamate fungicides around the world is Mancozeb (MZ). It is used in domestic, agricultural, and industrial applications which can increase its toxic effects. *Lemongrass* essential oil (LGEO) offers a broad scope of health benefits. This experiment aimed to investigate LGEO role in reducing renal toxicity induced by MZ in rats. **Material and Methods:** Seventy-five adult male albino rats were equally and randomly allotted into five groups (15 rats/group): The control groups (negative and vehicle), the *Lemongrass* group received LGEO 200 mg/kg, the MZ-treated group received 750 mg/kg MZ, and the combined group received LGEO with MZ as same doses mentioned before. The animals were treated orally for 8 weeks. **Results**: MZ Administration revealed a significant rise in serum creatinine, blood urea, and renal tissue malondialdehyde (MDA) levels, while superoxide dismutase (SOD) activities and glutathione content levels were significantly reduced in comparison to the control group. Also, the treated group with MZ revealed increased DNA damage and altered kidney histopathology, increased collagen deposit, with high expression of TNF-α, 8-OHdG, and p53 in renal tissue, as well as a high score of congestion, haemorrhage, infiltration, and necrosis. The rats treated with LGEO and MZ demonstrated partial improvement based on comet assay, renal oxidative stress parameters, and histopathological and immunohistochemical investigations. **Conclusion**: LGEO administration attenuates MZ induced renal injury via modulation of their oxidant, inflammatory, and apoptotic impacts. **Recommendation**: encourage use of safety measures as well as LGEO consumption among farmers to reduce MZ health consequences.

Keywords: *Mancozeb; dithiocarbamate; Lemongrass essential oil; GC-MS*

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INTRODUCTION

Pesticides are vital biochemical agents in controlling or killing pests. They are the utmost efficient way to reduce pests and raise crop production. Nevertheless, they have harmful environmental consequences, and are related to higher risk of human health hazards ***(Sule et al., 2022)***.

Mancozeb (MZ), a compound of ethylene bisdithiocarbamate class of fungicide, is one of the most efficient and extensively used agricultural pesticides. Furthermore, humans could be exposed to it by accidental consumption of mancozeb-contaminated agricultural products, water, or food chains. Their harmful impacts have been well recognized in individuals, involving the nervous system and genotoxicity ***(Aprioku et al., 2023)***.

Mancozeb is absorbed from the digestive system, dispersed to different organs, and excreted after 96 hours ***(Yahia et al., 2019)***. The toxic effects of MZ may be ascribed to its metabolite; ethylenethiourea, the latter has a long persistence in the earth or is quickly disintegrated in water, liberating potential toxic products like ethylenethiourea and propylenethiourea ***(Saber et al., 2019; Abdelkader et al., 2023a)***.

Mancozeb induces tissue injury through free radical activation and antioxidant inhibition**.** The most apparent mechanism of MZ-induced male reproductive organ toxicity in mice is oxidative stress induction, which provokes

apoptosis and chromosomal aberrations ***(Rašković et al., 2015; Srivastava et al., 2016; Girish and Reddy, 2018)***.

Cymbopogon citratus, an ornamental plant, is commonly used as a cooking and therapeutic plant. The oil derived from this plant is familiar for its medicinal properties, both pharmacological and *mental* ***(Soares et al., 2022; Fatunmibi et al., 2023)***.

Lemongrass essential oil (LGEO) has a noteworthy amount of several bioactive composites, like citral, isogeranial, isoneral, geranyl acetate, citronellol, and germacrene-D …etc. These constituents have diverse pharmacological actions, including antimicrobial (viral, bacterial, and fungal) and antioxidant possessions ***(Mukarram et al., 2021; Abdelkader et al., 2023b)***.

Aim of the study

THE AIM OF THE WORK

The aim of the current experiment was to investigate the potential benefits of using LGEO to mitigate the harmful effects of Mancozeb on kidney in rats. The results of this research could shed light on beneficial effects of LGEO to protect damaged kidneys via modulation of MZ induce oxidant, inflammatory, and apoptotic impacts.

MATERIAL AND METHODS

Mancozeb: Dithane M-45 (Mancozeb 80% wettable powder) was gotten from Dow Agro Sciences Co., France.

Lemongrass essential oil: It was gained by hydro-distillation of the organic *Cymbopogon citratus* plant which was obtained from the local garden.

Fresh *Lemongrass* was gained from the Medicinal and Aromatic Plants Research Department's farm in El Kanater El Khairia, Egypt. The samples were prepared and dried in an oven and saved in a closed bag, then, through steam distillation equipment, the oil was condensed and separated from the sample ***(Amenaghawon et al., 2014)***.

LGEO was analyzed using gas chromatography-mass spectrometry (GC-MS) in the Zagazig Forensic and Clinical Toxicology Research Lab, Faculty of Medicine, Zagazig University according to ***Abd El-Kareem et al. (2016)***.

GC-MS analysis of *Lemongrass* essential oil: Analysis of LGEO by GC-MS, there are different compounds, fifteen in number, as presented in **Table (1) and Figure (1)**.

Table (1): Major compounds identified in LGEO showing their names, retention time, molecular formula, molecular weight, and area percent.

RT	Compound Name	Molecular Formula	Molecular Weight	Area %
6.88	Eucalyptol	C10H18O	154	1.76
11.65	2,6-Octadienal, 3,7-dimethyl-, (Z)-	C10H16O	152	20.94
11.92	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	C10H18O	154	4.12
12.33	2,6-OCTADIENAL, 3,7-DIMETHYL-, (E)-	C10H16O	152	31.12
13.14	1,2-BENZENE DICARBOXYLIC ACID, MONOMETHYL ESTER	C9H8O4	180	6.09
13.30	1,2-Benzenedicarboxylic acid	C8H6O4	166	3.34
13.44	1,2-Benzenedicarboxylic acid	C8H6O4	166	4.36
14.30	Geranic acid	C10H16O2	168	3.26
14.81	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	C12H20O2	196	2.78
17.80	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3 -yl ester	C16H26O2	250	3.46
18.69	DODECANOIC ACID	C12H24O2	200	8.82
19.90	Cedran-diol, 8S,13-	C15H26O2	238	2.89
20.66	CYCLOPROPANEBUTANOIC ACID, 2-[[2-[[2-[(2-PENTYLCYCLOPRO PYL)METHYL]CYCLOPROPYL] METHYL]CYCLOPROPYL]METH YL]-, METHYL ESTER	C25H42O2	374	3.40
21.58	1-Heptatriacotanol	C37H76O	536	2.15
21.75	ALANINE, 3-(BENZYLOXY)-, L-	C10H13NO3	195	1.50

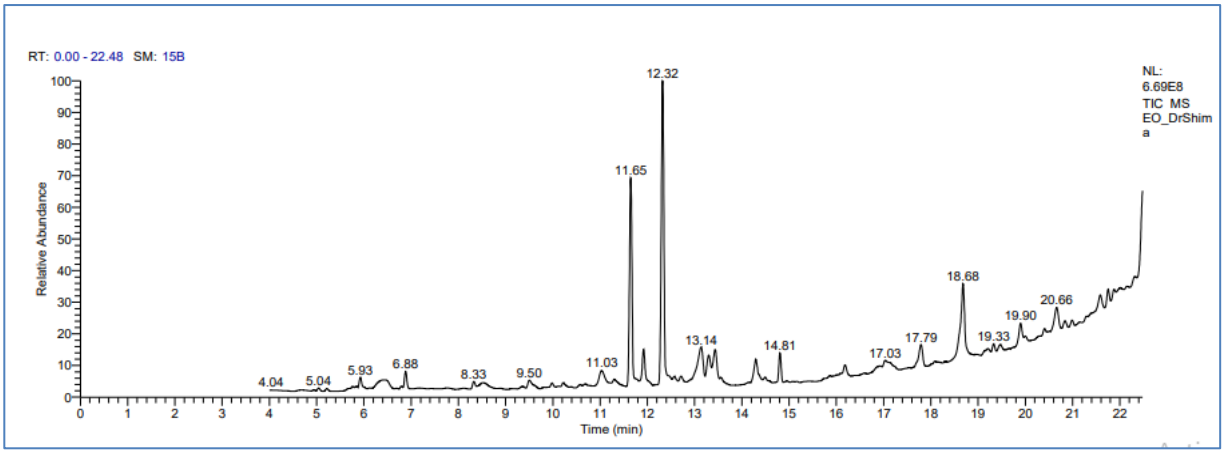


Figure (1): Total ionic chromatogram (GC-MS) of Lemongrass essential oil

Animals and Experimental Design

Seventy-five adult male albino rats, weighing about 180–200 gm, were included in the experiment that steered in the animal house of the Faculty of Medicine, Zagazig University. Before the start of the experiment, all rats were exposed to seven days intervals of impassive preliminaries to acclimatize rats to the new setting, to establish their health, and to prohibit any sickness. They received well-adjusted food, rich in all nutrients, required for their health throughout the experiment. It included bread and barley. Water was present in isolated clean suppliers. All experimental procedures were done according to the Institutional Animal Care and Use Committee guidelines, Zagazig University, Egypt (Ethical approval number ZU-IACUC/3/F/89/2021).

The animals were equally and at random allotted into 5 groups (15 animals/group) that were separately caged: all treatments were gavaged orally for 8 weeks duration.

- **Group I: (Negative control):** every rat received a regular diet and tap water to establish the basic values of performed tests.
- **Group II: (vehicle control)**: each rat gavaged 1mL/day of corn oil (as a vehicle for dissolving both the *Lemongrass* essential oil and mancozeb)
- **Group III: (LGEO-treated):** each rat received daily Lemongrass essential oil at a dose of 200 mg/kg B.W dissolved in corn oil ***(Li et al., 2018)***.
- **Group IV: (MZ-treated):** each rat received daily 750 mg/kg B.W of MZ (1/20 of LD_{50}) dissolved in corn oil.

The LD_{50} of oral administration of MZ in rats is 15000 mg/kg ***(Kackar et al., 1997)***.

- **Group V: (LGEO+MZ-treated):** each rat received daily both LGEO and MZ with the same previous doses.

All groups were carefully observed for any mortality that might occur because of substance administration. Dosages of each substance administered were freshly prepared daily and adjusted weekly according to body weight changes. By the end of the experiment (24 hours from the last treatment) 2 mL of blood sample, from each anesthetized animal, was obtained from the retro-orbital plexus into a clean centrifuge tube and kept until blood clotted (incubated at 37°C) then centrifuged to separate the serum (for 10 minutes at 3000 r.p.m) then subjected to the biochemical studies, which were assessed at Biochemistry Department, Faculty of Medicine, Zagazig University. Then, the rats were euthanized, and both kidneys were dissected from each rat and examined to detect any gross abnormalities then bathed with saline and dried; the right ones were homogenized in saline by a homogenizer, and after that, they were used for oxidative stress biomarkers assessment and DNA damaged test. The left ones were used for histopathological and immune-histochemical studies.

Renal Function Analysis

Stored serum samples were used for measurement of the serum creatinine level (mg/dL) and blood urea level (mg/dL). This was done according to the manufacturer's

instructions using colorimetric diagnostic kits (Bio Diagnostic, Egypt)

Oxidative Stress Markers Analysis

The levels of malondialdehyde (MDA) (nmol/mg tissue), Superoxide dismutase (SOD) (U/mg tissue), and Reduced glutathione (GSH; ng/mg tissue) were assessed using kits from Bio-diagnostic, Cairo, Egypt.

DNA Damage Tests in Renal Tissues (Single-Cell Gel Electrophoresis)

The comet assay was done at the Animal Reproductive Research Institute of Agricultural Research Centre-Ministry of Agriculture and Land Reclamation (Elharam, Giza); the method was described *by* ***Khan et al. (2015)***.

Histopathological and Immunohisto chemical Examination

The left kidneys were cut into small pieces for histopathology and then fixed in 10% buffered formalin. Tissue samples were prepared for paraffin-embedding and consecutive sections (5 µm thickness) were obtained for hematoxylin and eosin (H&E) staining to enable routine histological examination. They were also stained by Masson–Trichrome to evaluate the presence of collagen fibers in renal tissues ***(Bancroft and Layton, 2013)***. A pathologist evaluated ten fields from each group at a magnification of X400 to identify signs of degeneration, congestion, infiltration, and hemorrhage. Based on a grading checklist, these changes were evaluated and graded for each microscopic field from 0 to 4+ (0 indicating no abnormalities and 1-4 indicating mild to severe changes) ***(Küçükler, et al., 2021)***. For immunohistochemical staining, paraffin slices were used with rabbit polyclonal antibodies against the inflammatory marker Tumor necrosis factor α (TNF-α) ***(Taal et al., 2000)***, the oxidative stress marker 8-hydroxy-2-deoxyguanosine (8-OHdG) ***(Taguchi et al., 2012)***, and apoptosis marker P53 ***(Khamis et al., 2023)***. The kits used for the staining were supplied by DAKO Life Trade Egypt. Image J analysis software (NIH, USA) was used to assess the intensity (%) of collagen fibers (in Masson-stained sections), TNF-α (in TNF-α stained sections), 8-OHdG (in 8-OHdG stained sections), P53 (in P53 stained sections) and counting the apoptotic cells number (in P53 stained slices) in five consecutive sections from slides of every rat in all groups. By using a light microscope LEICA DM500, slides were examined and photographed in the Anatomy Department, Faculty of Medicine, Zagazig University.

STATISTICAL ANALYSIS:

Data were collected and presented as the mean ± SD and median, and Interquartile Range (IQR). One-way analysis of variance (ANOVA) was used for data analysis, followed by least significant difference for multiple group comparison. While, Kruskal Wallis test was used for comparison between more than two different groups of quantitative data which were not normally distributed. P-value<0.05 denoted statistical significance using the statistical software package SPSS for Windows (Version 20; SPSS Inc., Chicago, IL, USA).

RESULTS

Results of negative and vehicle control groups were within the normal range, and no statistically significant difference was observed ($p > 0.05$). So, the negative control group's results were used as a reference for comparison with the treated groups across all the measured parameters.

1- Effect on the Kidney Functions

After 8 weeks of administering MZ, it was observed that there was a significant rise in the levels of serum creatinine and blood urea, in comparison with the control, with (P-value < 0.001). However, when LGEO was co-treated with MZ, there was a significant reduction in the levels of kidney function (P-value <0.001), in comparison with animals that were treated with MZ alone **(Figure 2).**

2- Effect on Renal Oxidative Stress Markers

After being administered MZ for eight weeks, the renal MDA levels in rats were found to be significantly raised ($P < 0.001$) in comparison with the control, indicating the induction of lipid peroxidation. Moreover, in MZ-treated rats, there was a marked reduction in tissue antioxidants; the levels of GSH contents (non-enzymatic) and SOD activities (enzymatic) as compared to the control. Conversely, co-administration of LGEO with MZ, a significant drop (P <0 .001) in oxidative stress was observed **(Figure 3).**

3-Assessment of DNA Damage in Renal Tissue (COMET Assay)

The cellular appearance of the comet assay was observed in control (**Figure 4a**) and LGEO groups (**Figure 4b**) on a microscopic level, which indicated that there was no DNA damage because the cells did not have tails. After the cells were treated with MZ, the comet assay revealed elongated tails in the damaged DNA of the cells (with longer tails indicating more damage) (**Figure 4c**). This resulted in a significant rise ($P < 0.001$) in the percentage of Tail DNA and Tail length (um) as compared to the control, as depicted in **Figure (4e** and **f)**. However, when LGEO was co-administered with MZ, the length of the DNA tails decreased, indicating an improvement in the level of DNA damage, as seen in **Figure 4d**. There was also a substantial decrease in Tail DNA % and Tail length compared to the MZ group (**Figure 4 e and f).**

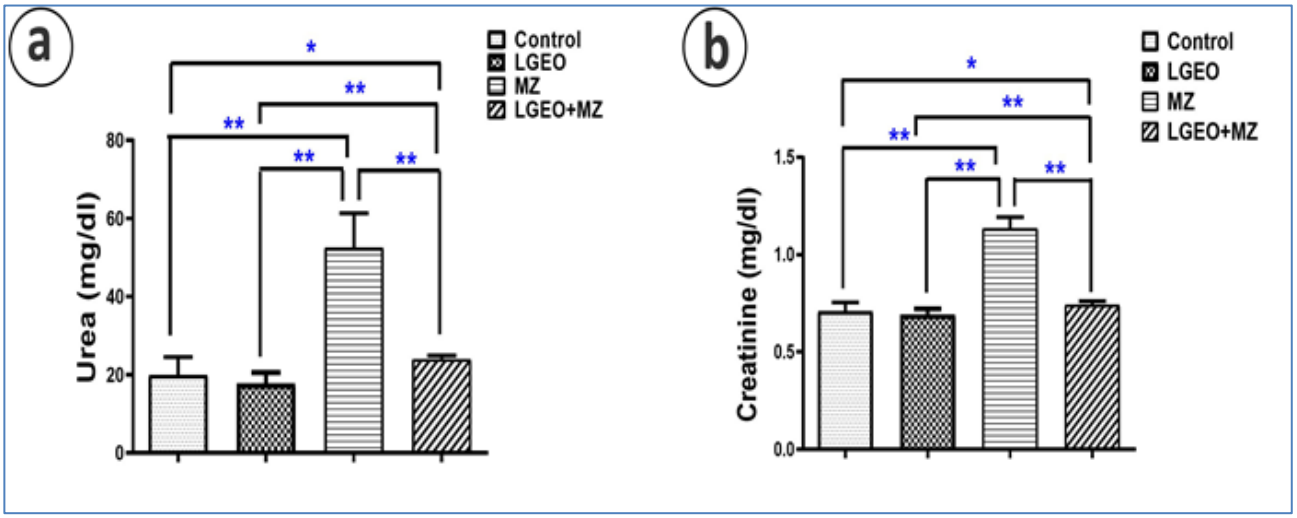


Figure (2): a) Blood urea level (mg/dL). b) Serum creatinine (mg/dL) in different studied groups. Mean ± standard deviation (SD) of n = 15 rats/group. * P <0.05, significant; * *P <0 .001, highly significant.

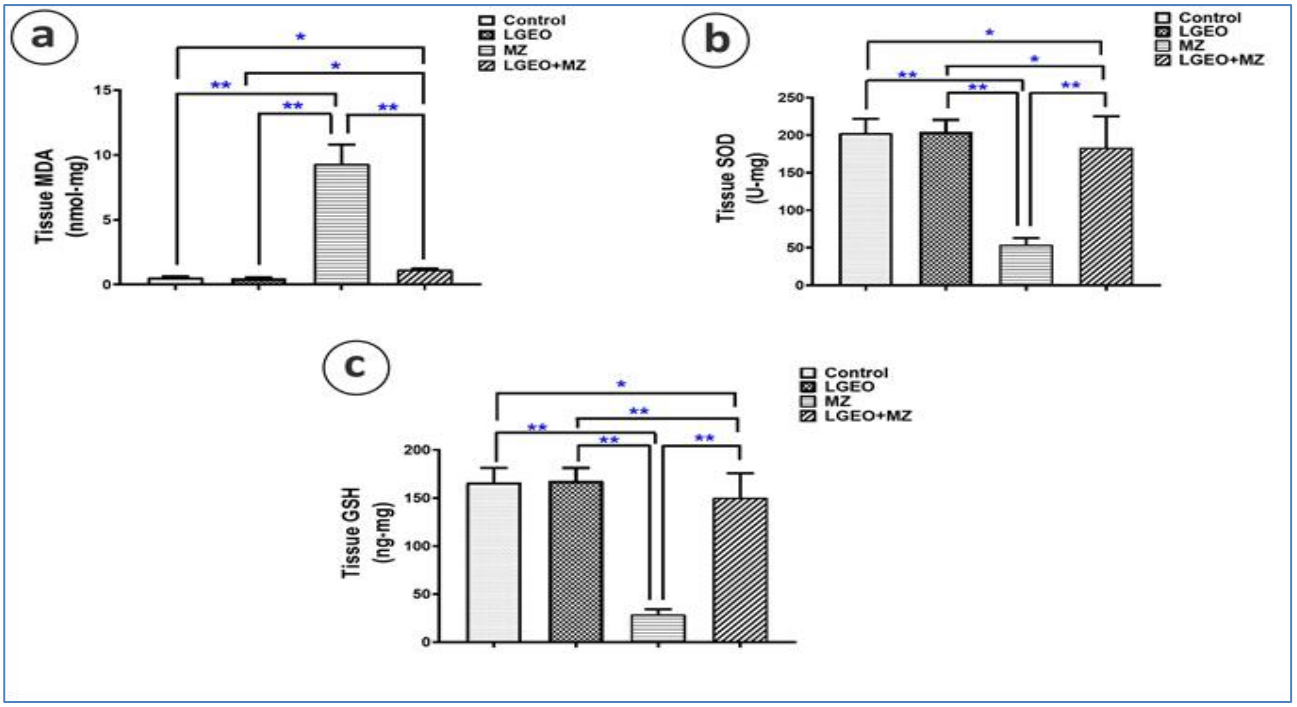


Figure (3): a) Tissue MDA (nmol/mg). b) Tissue SOD (U/mg) c) Tissue GSH (ng/mg) in different studied groups. Mean ± standard deviation (SD) of n = 15 rats/group. * P <0.05, significant; * *P <0 .001, highly significant.

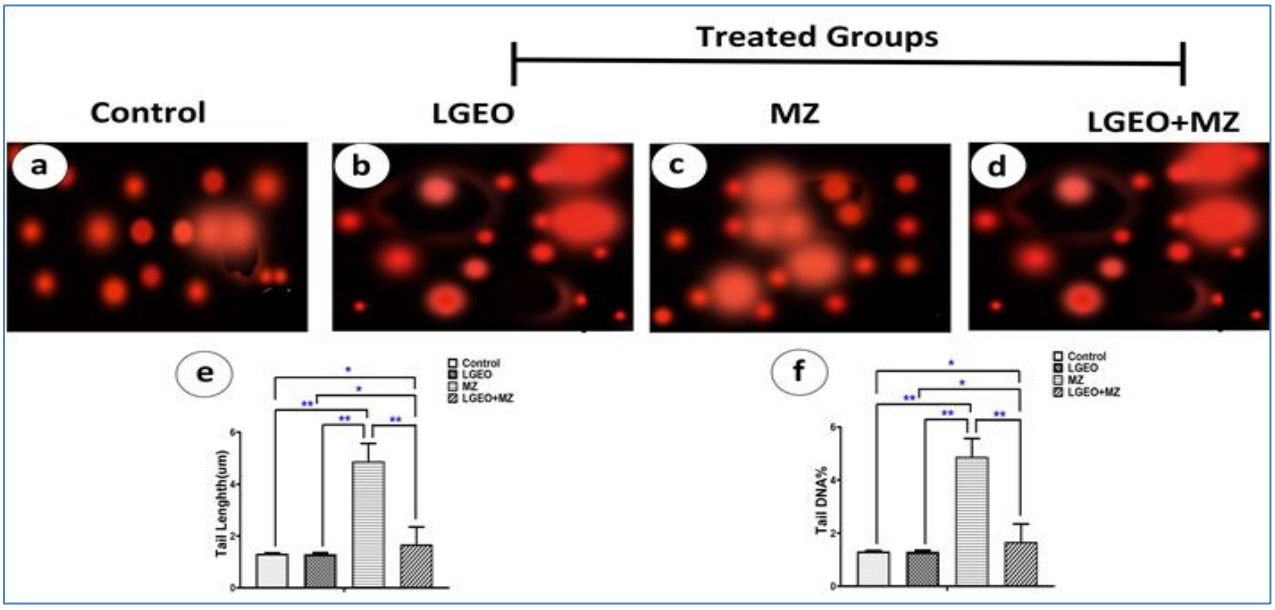
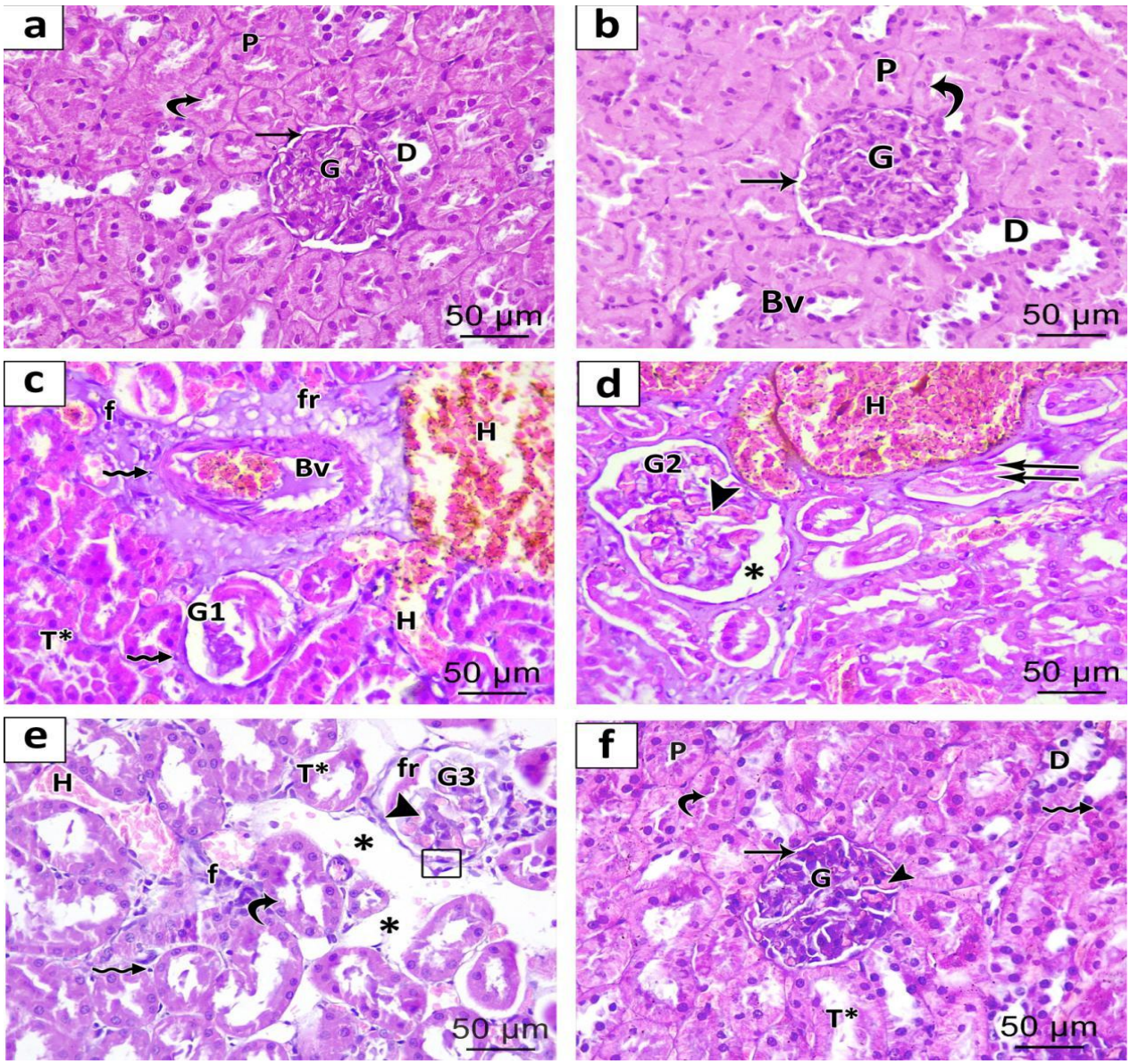


Figure (4): Comet assay in renal tissue in different studied groups; a) control b) LGEO-treated group with no DNA tails indicating no DNA damage. c) The MZ-treated group showed long DNA tails Indicating considerable DNA damage. d)* LGEO+*MZ -treated group showed little DNA tails indicating less DNA damage. e) tail length (µm), f) Tail DNA %. Mean ± standard deviation (SD) of n = 15 rats/group. * P <0.05, significant; * *P <0 .001, highly significant.

4- <u>Effect on Histopathological and Scoring Results</u>

Light microscopic examination of renal cortex sections, H & E stained, from control and LGEO groups showed a normal histological architecture regarding corpuscles and tubules. Every corpuscle comprised of a glomerulus having capillaries tuft and was encircled by Bowman's capsule that was parted by Bowman's space. The cortical renal tubules were formed principally of proximal and distal convoluted tubules (**Figure 5a and b**). Examination of the kidney sections from the group treated with MZ revealed one glomerulus that had collapsed with a wide renal capsular space. In other sections, some glomeruli showed fibrosis and lobulation with a thickened capsule. There were several renal tubular injuries in the form of degeneration and loss of brush border. The interstitium had severe interstitial hemorrhage, fibrosis, inflammatory cell infiltration, and congested blood vessels resulting in widening. These observations are shown in **Figure (5c, d, and e).** Administration of LEGO with MZ led to a partial improvement in the histological structure of the renal cortex. The glomeruli showed a better morphology in comparison with the MZ group, except for focal congestion of glomeruli and some pyknotic cells in the wall of tubules. This is depicted in **Figure (5f)**.

The Masson's trichrome stain showed normal distribution of collagen in control and LGEO-treated rats **(Figure 6a and b)**. Rats treated with MZ showed significant collagen accumulation in the peritubular zones of the cortex, brush borders of the proximal tubules, periglomerular matrix, and mesangial matrix, and thick blood vessel walls **(Figure 6c)**. Co-administration of LGEO with MZ resulted in lower collagen deposition **(Figure 6d)**. This was proved statistically by comparing the area% of Masson's trichrome staining among various groups, with no significant difference between control and LGEO groups, a significant rise in MZ group, and a significant reduction when LGEO co-administered with MZ **(Figure 6e)**. The histopathological scoring shown in **Table (2)** revealed a vastly significant difference among the different groups; the renal sections in the MZ-treated group showed destructive changes with high scoring of congestion, hemorrhage, infiltration, and necrosis. However, the rats treated with LGEO with MZ showed moderate improvement in renal cortical tissues and



pathological scoring of hemorrhage and infiltration.

Figure (5): *Photomicrographs of rat renal cortex in the* ***(a):*** *control and* ***(b):*** *LGEO-treated groups showing proximal convoluted tubules (**P**) with apical brush border (**curved arrow**) and distal convoluted tubules (**D**). A narrow interstitium with a small blood vessel (**Bv**) is noticed. Renal corpuscles with a glomerular tuft (**G**) and narrow Bowman's space (**arrow**) are observed.* ***(c, d and e):*** *MZ-treated group, presenting severe glomerular disruption in the form of necrosis (**G1**), marked lobulation (**G2**), and fibrosis (**G3**). The glomerular capillaries showing marked congestion (**arrowheads**); while Bowman's capsule revealed thickening (**boxed area**) and space showed widening (*). The cortical tubules (**T***) display marked degeneration (**double arrows**). Other tubules show pyknotic cells (**zigzag arrows**) in their walls with loss of brush borders (**curved arrow**). The interstitium showed widening in some areas (*), a large area of hemorrhage (**H**), fibrosis (**fr**), dilated congested blood vessel (**Bv**) with a thickened wall, and inflammatory cell infiltration (**f**).* ***(f):*** *LGEO with MZtreated group showing moderate improvement; renal corpuscles with a glomerular tuft (**G**) with focal congestion of glomeruli (**arrowhead**) and Bowman's space (**arrow**). Some proximal convoluted tubules (**P**) show intact brush border (**curved arrow**) and distal convoluted tubules (**D**)restore their structure except some distorted tubules(**T***) are still observed. Few pyknotic cells (**zigzag arrow**) still appear in the wall of tubules.* ***Scale bar = 50 µm, X400***

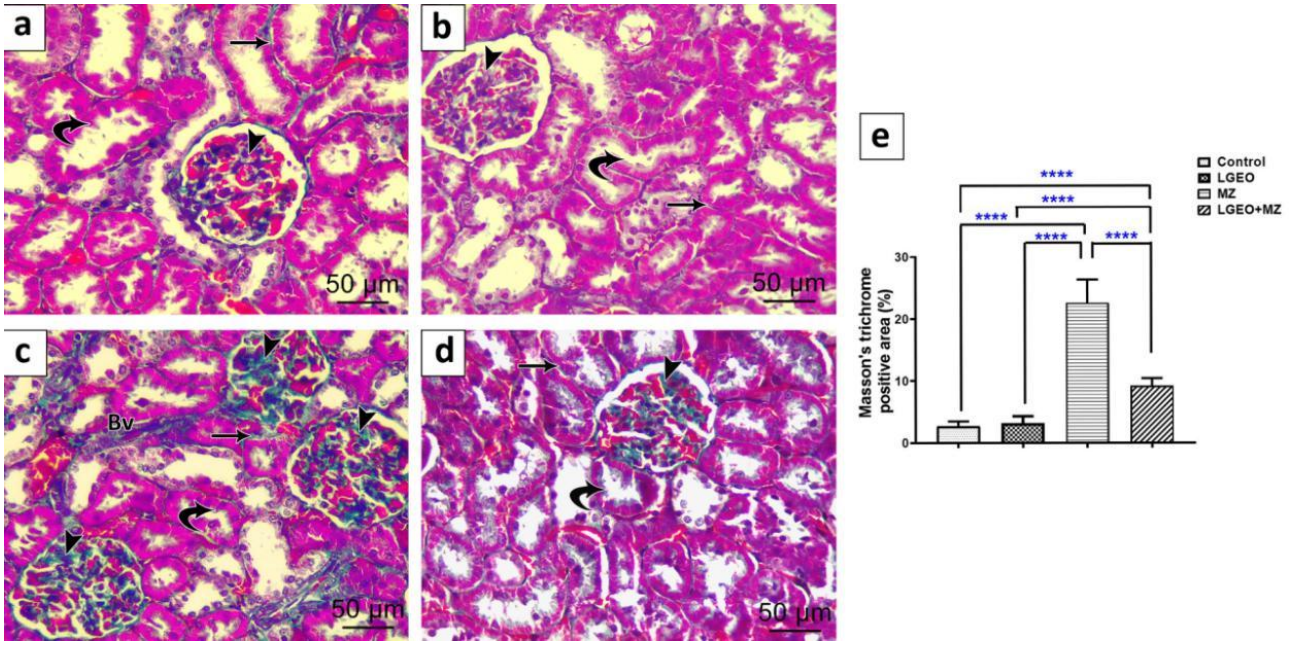


Figure (6): *Representative Images showing Masson's trichrome stained renal cortex sections in different experimental groups:* ***(a)*** *control,* ***(b)*** *LGEO, (c) MZ* ***(d)*** *LGEO+MZ-treated groups. Arrows in the peritubular areas, curved arrows in brush borders of the proximal convoluted tubules, and arrowheads in the mesangial matrix indicate collagen distribution in different experimental groups.* ***(e)*** *Intensity of renal Masson's trichrome (% area). Data are expressed as means ± SD. **** indicates a significant difference (p < 0.05).* ***Scale bar = 50 µm, X400***

Table (2): Scoring of Histopathologic changes in rat kidneys of the different studied groups.

Damage score	Negative Control group	LEGO-treated group	MZ-treated group	LEGO+MZ-treated group
Congestion (median (IQR))	**0.0(0.0)**	**0.0(0.0)**	**3(1)^{a,b}**	**1(1)^{c}**
Hemorrhage (median (IQR))	**0.0(0.0)**	**0.0(0.0)**	**3(0)^{a,b}**	**1(0.25)^{c}**
Necrosis (median (IQR))	**0.0(0.0)**	**0.0(0.0)**	**3(1)^{a,b}**	**0.5(0.5)^{c}**
Infiltration (median (IQR))	**0.0(0.0)**	**0.0(0.0)**	**2.5(0.5)^{a,b}**	**0.5(.5)^{c}**

Note: Values are expressed as median and Interquartile Range (IQR) of eight rats in each group. Different superscripts (a–c) in the same row indicate significant difference among each group (p < .05). a: significant versus control group at p<0.05, b: significant versus LEGO-treated groups at p<0.05 c: significant versus MZ-treated group at p<0.05. Abbreviations: LEGO; Lemongrass-treated; MZ, Mancozeb-treated; LEGO+MZ, Lemongrass+ Mancozeb- treated groups.

5-<u>Effect on Renal Immunohistochemistry of TNF-α, 8-OHdG, and P53</u>

Immunohistochemistry of TNF-α in control and LGEO-treated groups revealed a poor immune response in the cytoplasm of cortical tubules **(Figure 7a and b)**. Rats treated with MZ only revealed a high immunohistochemical expression of TNF-α in harmed and degraded renal tubular epithelial cells **(Figure 7c)**. While co-treatment of LGEO with MZ caused a significant improvement in the kidney tissues' responses to TNF-α compared to MZ group **(Figure 7d).** The mean area % of TNF-α expression was noticeably greater in renal sections of the

MZ-treated group as compared to the control and LGEO+MZ groups (**Figure 7e).**

In the cortical tubules, the immune response to 8-OHdG was weak, and the immunological reaction was negative in the cytoplasm of renal corpuscles for both the control and LGEO groups **(Figure 8a and b)**.

In the case of MZ-treated rats, the immunohistochemical expression of 8-OHdG was high in the harmed and damaged renal tubular epithelial cells **(Figure 8c)**.

However, when MZ and LGEO were administered to the animals, a significant improvement was observed in the kidney tissue's responses to hydroxydeoxyguanosine compared to MZ alone **(Figure 8d**). The renal sections of the MZ group revealed a noticeably greater mean area % of 8-OHdG expression compared to the control group, while the LGEO+MZ group revealed a partial improvement **(Figure 8e)**.

Immunohistochemistry of P53 revealed a weak immunoreaction in the control and LGEO groups **(Figure 9a and b)**. Both glomeruli and tubular cells in the MZ-treated group displayed strong p53 immuno-expression; however, the immunostaining was more pronounced in the tubular cells **(Figure 9c)**. Additionally, the glomeruli and tubular ones of the LEGO+MZ-treated group expressed moderate p53 immunoreactivity **(Figure 9d)**. Concerning the average area % of P53 expression and the P53 immuno-positive cells number per section was noticeably greater in the MZ-group in comparison with the control and the combined groups shown in **figure (9e and f)**.

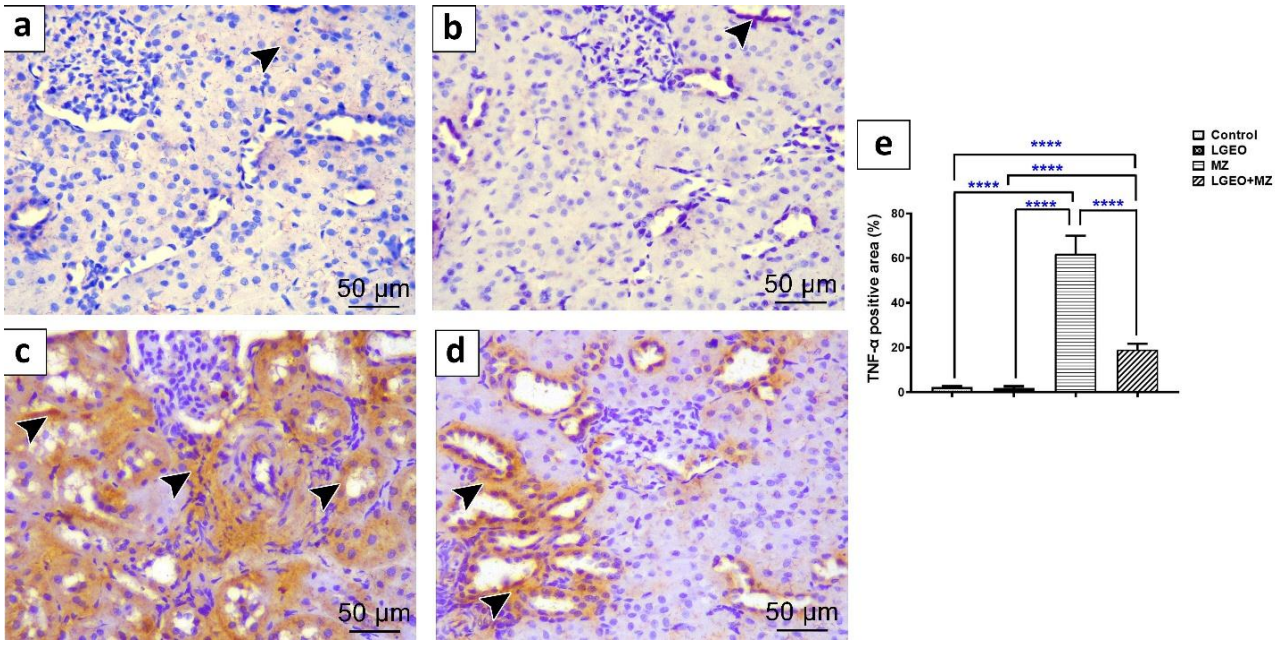


Figure (7): *Representative Images showing TNF-α immuno-positive cell expression,* ***(a)*** *control,* ***(b)*** *LGEO,* ***(c)*** *MZ* ***(d)*** *LGEO+MZ-treated groups. Arrowheads indicate a positive immune reaction in the cytoplasm of the tubular cells of different studied groups.* ***(e)*** *Immunostaining intensity of renal TNF-α (% area). Data are expressed as means ± SD. **** indicate a significant difference (p < 0.05). Scale bar = 50 µm, X400*

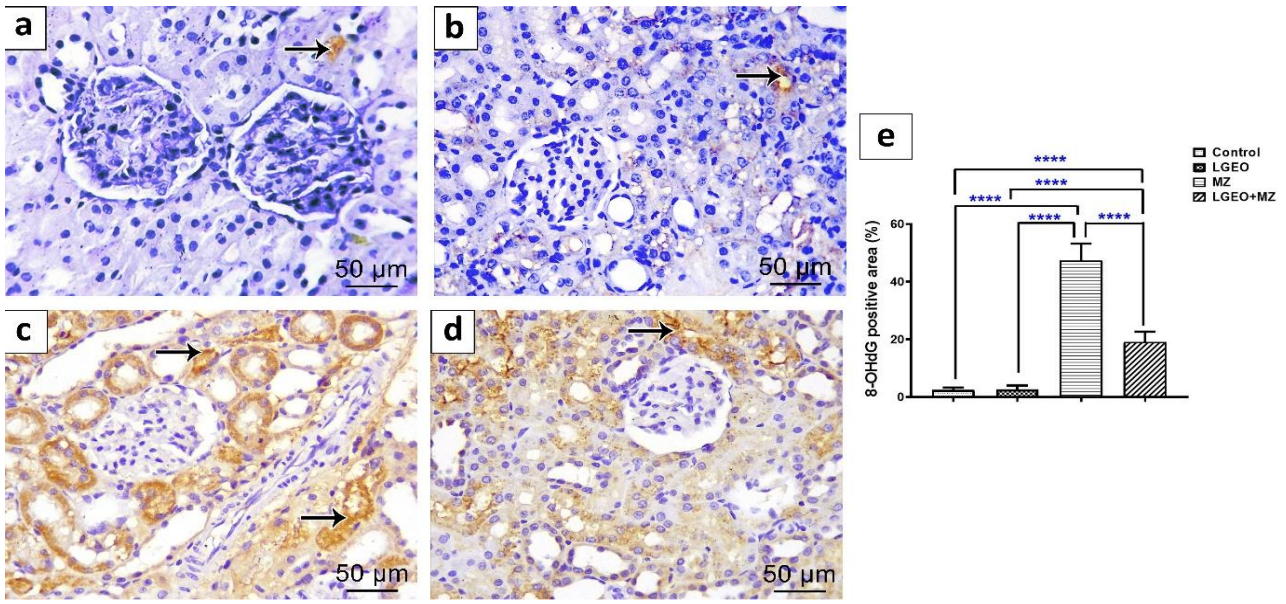


Figure (8): *Representative Images showing 8-OHdG immuno-positive cell expression,* ***(a)*** *control,* ***(b)*** *LGEO,* ***(c)*** *MZ* ***(d)*** *LGEO+MZ-treated groups. Arrows indicate a positive immune reaction in the cytoplasm of the tubular cells of different studied groups.* ***(e)*** *Immunostaining intensity of renal 8-OHdG (% area). Data are expressed as means ± SD. **** indicate a significant difference (p < 0.05).*

Scale bar = 50 µm, X400.

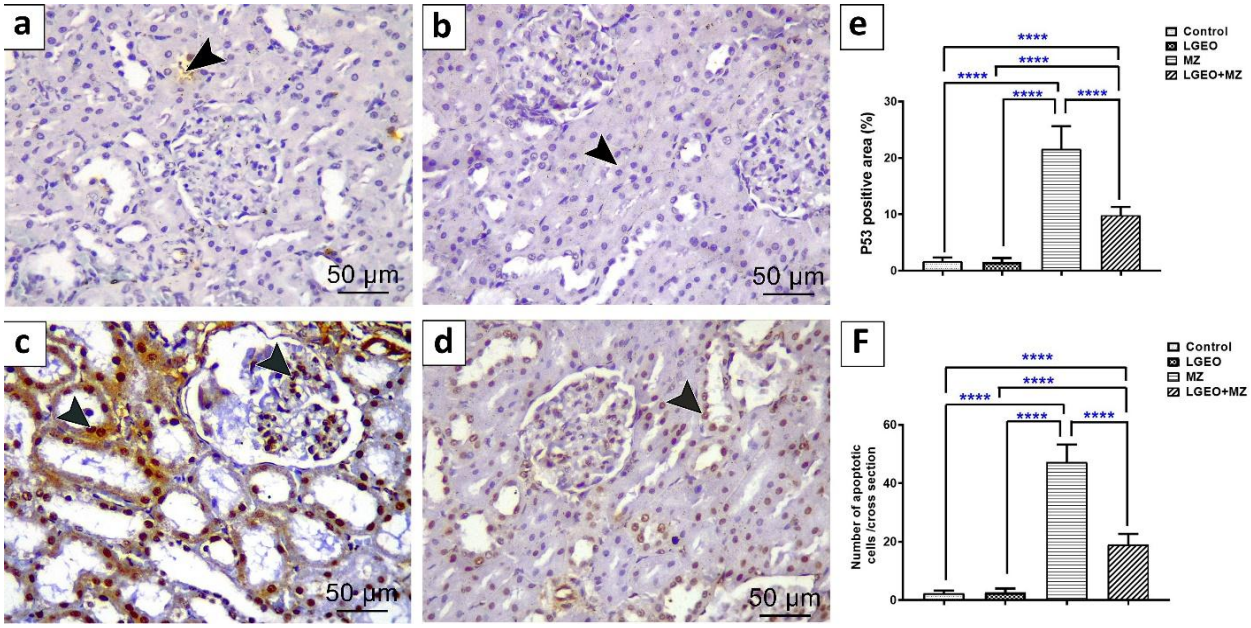


Figure (9): *Representative Images showing P53-immuno-positive cell expression,* ***(a)*** *control,* ***(b)*** *LGEO,* ***(c)*** *MZ* ***(d)*** *LGEO+MZ-treated groups. Arrowheads demonstrate dark brown staining of immune-positive nuclei of apoptotic cells in tubular and glomerular cells.* ***(e)*** *Immunostaining intensity of renal P53 (% area).* ***(f)*** *Number of apoptotic cells per crossed section. Data are expressed as means ± SD. **** indicates a significant difference (p < 0.05).* *Scale bar = 50 µm, X400.*

DISCUSSION

Mancozeb is a fungicide that farmers heavily depend on to safeguard crops from a variety of fungal diseases that commonly occur in agriculture. As a result, MZ and its byproducts are extensively present in the environment, and their potential toxicity can pose a risk due to the likelihood of exposure. In addition, LGEO is known for its prominent flavonoid and phenol constituents, moreover, it is accessible in diverse nations and has medicinal and industrial benefits. According to the present research, it has been found that LGEO can be an effective therapeutic agent in shielding rats from renal damage caused by MZ.

In the present research, the increased serum creatinine and blood urea were observed in MZ-treated rats at a dose of 750 mg/kg/day for 8 weeks compared to the control group. These results were supported by ***Yahia et al. (2015)*** and ***Kotb et al. (2019)*** who stated a significant upsurge in serum levels of creatinine and blood urea in MZ-treated rats.

The elevated creatinine and urea levels are indicators of critical injury to the nephron's structural integrity; urea is a byproduct of protein metabolism. The body's reliance on the kidneys to excrete urea makes it a valuable indicator to assess their function ***(Salazar, 2014)***.

Creatinine, an amino acid, is developed from the nonenzymatic transformation of creatine in muscular tissue then underwent hepatic metabolism via methylation of guanidine amino acetic acid to form creatinine. It is undergoing the glomerular filtration and is not reabsorbed or metabolized by the kidney. Hence, creatinine clearance is the utmost common marker for assessing glomerular filtration rate ***(Ronco et al., 2010)***.

Kidney function impairment by MZ was confirmed by the histopathological findings, including some collapsed glomeruli, and degeneration of renal tubules with hemorrhage, fibrosis, inflammatory cell infiltration, and congested blood vessels in the interstitium. In addition, increased collagen deposition surrounding the periglomerular, peritubular, and mesangial matrix and thicker congested blood vessel walls were detected by using Masson's trichrome stain. These findings suggested that MZ induced cellular toxicity.

The current results were consistent with ***Talha and Abd-Allah's*** study in ***(2006)*** that revealed oral administration of MZ caused several histopathological abnormalities in rats, including degeneration in the renal tubules and congestion of blood vessels, focal congestion in glomeruli, interstitial hemorrhage, and inflammatory cells. These results were confirmed by scoring.

In line with the findings of ***Sakr and Shalaby (2012)***, animals that were subjected to metiram, a dithiocarbamate fungicide, exhibited degeneration of renal tubules, shrinkage of glomeruli, and infiltration of leucocytes between the tubules. The epithelium lining the renal tubules displayed signs of cloudy swelling and vacuolated cytoplasm with pyknotic nuclei. The changes in kidney histology may have been caused by a reduction in antioxidants and rise in lipid peroxidation.

Zhang et al. (2023), also found that MZ-treated rats had minimum lymphocyte infiltration in the renal medulla and tubular atrophy, minor degeneration of the tubular epithelial capsule, and glomerular injury.

Lowering the levels of creatinine and urea to normal was noted when LGEO was co-treated with MZ, indicating the prevention of nephron toxicity. In addition, Co-administering LGEO and MZ to rats resulted in partial improvement of histopathological changes, including reduced collagen deposition caused by MZ, in the current study which aided the kidneys in maintaining their normal function and reducing the increase in creatinine and urea levels.

The findings of ***Arhoghro and Kpomah (2013); Ullah et al. (2013); Said et al. (2019) and Fahmy et al. (2020)***, supported the results of the current research and demonstrated that Cymbopogon citratus co-treatment successfully reduced renal damage caused by cisplatin, aminoglycosides, adenine, and carbon tetrachloride, respectively, by improving renal function parameters (urea and creatinine) and histological changes in the renal tissue. The antioxidant properties and strong flavonoid

content of Lemongrass could contribute to its renal protective effect.

Several mechanisms might clarify MZ-induced renal toxicity; the study results showed that MZ-induced oxidative stress as imitated by a significant upsurge in MDA, and a diminution in non-enzymatic (GSH) as well as enzymatic (SOD) antioxidants in renal tissue of MZ-treated rats as compared to the control. These results were like earlier studies; ***Sakr and Saber (2007); Hashem et al. (2018) and Mohammadi-Sardoo et al. (2018)***, reported a significant upsurge in MDA with a significant decline in GSH level and SOD activity and catalase designating raised oxidative stress with subsequent apoptosis in MZ treated rats.

SOD, an antioxidant enzyme, is considered as the first line of defense against free radicals. Consequently, declined activity of it indicates the failure of the prime antioxidant system to act against reactive oxygen species, and this may be attributed to one of the following mechanisms, The first one could comprise the SOD exhaustion in breakdown the free radical produced by MZ or the hang-up of the SOD by them. The second one could be the direct SOD hang-up by MZ. In this study, MZ also triggered a substantial reduction in GSH level, which is one of the utmost imperative ROS scavengers (intracellular and extracellular), which may be ascribed to the augmented utilization of GSH to scavenge and neutralize ROS produced ***(Zitka et al., 2012; Balaji et al., 2014)***.

In the current experiment, co-treatment of LGEO and MZ revealed a significant reduction in MDA levels and a significant raise in GSH and SOD levels in renal tissue compared to MZ treatment alone. Lemongrass extract has been informed to have antioxidative activities and the ability to reduce MDA concentration and increase GSH levels, which prevented paracetamol-induced damage, according to ***Saenthaweesuk et al. (2017)***. The reduction in MDA production and elevation in antioxidant activities of lemongrass were attributed to its phenolic compounds, as stated by ***Somparn et al. (2018)***.

Administration of MZ in the current research yielded a substantial rise in tail DNA percentage and tail length, as compared to the control. ***Yahia et al. (2019)*** observed a significant rise in DNA damage index and tail length in the colon and hepatic tissues of MZ treated rats.

Additionally, in-vitro experiments have established the genotoxic impact of MZ, as it significantly increased tail length and tail moment, indicating that MZ could have an adverse impact on DNA integrity ***(Lori et al., 2021)***.

A significant decrease in Tail DNA % and Tail length was observed when LGEO was co-administered with MZ as compared to the MZ group. Previous studies have reported the protective effect of LGEO against DNA damage. For instance, ***Bidinotto et al. (2011)*** demonstrated that oral administration of LGEO reduced DNA damage in blood leukocytes of mice treated with cumulative N-methyl-Nnitrosurea.

El-Garawani (2015) investigated the antigenotoxic potential of Lemongrass leaves aqueous extract against total genomic DNA damage induced by cisplatin in human peripheral leukocytes and found promising results.

Furthermore, ***Fahmy et al. (2020)*** reported that LGEO has a significant antigenotoxic effect against carbon tetrachloride-induced chromosomal damage in the bone marrow and spermatocyte of treated mice by scavenging ROS.

The disruption in the balance between oxidants and antioxidants was correlated to a highly positive response to 8-OHdG immunohistochemical staining in damaged renal tubular epithelial cells in MZ-treated rats in current research.

The co-administration of LGEO and MZ resulted in a weak immunohistochemical response, indicating an improvement in the histopathological changes caused by MZ. The immune staining area percentages in renal tissues across different experimental groups verified all these findings. ***Küçükler et al. (2021)*** found a significant rise in 8-OHdG expression in rat kidney tissues treated with chlorpyrifos, which resulted in DNA damage in tubular cells of the kidney.

8-OHdG is a DNA adduct that is produced by hydroxylation of the deoxyguanosine residues

of DNA and it is considered one of the most common free radical-induced oxidative injuries. It is widely used as an oxidative stress biomarker in various experiments ***(Ock et al., 2012)***. In-vitro studies have suggested that MZ acts as a pro-oxidant leading to cellular oxidative stress by producing free radicals, DNA adducts, and 8-OHdG in rat cells ***(Tsang and Trombetta, 2007)***.

An experiment conducted by **Khabour et al. in 2023** examined the influence of LGEO on oxidative DNA damage in human lymphocytes. The study found that LGEO significantly lowered the level of 8-OHdG, indicating its defensive impact against DNA damage. The findings of the study were further endorsed by ***Koklesova et al. (2020)***, who confirmed that LGEO has the possibility to act as a chemo-preventive compound.

Also, MZ treated group revealed high immunohistochemical expression of TNF-α, an inflammatory marker, in damaged renal tubular epithelial cells, and a strong p53 immuno-expression, apoptotic marker which was more pronounced in the tubular cells.

Several studies suggest that MZ provokes apoptosis via both intrinsic and extrinsic pathways triggered by oxidative stress. In terms of the intrinsic pathway, alterations in the mitochondria's membrane potential and increased permeability allow apoptotic effectors to leak out, as described by ***Bao et al. (2022)***, and ***Zhang et al. (2023)***. As for the extrinsic way, TNF-α, a pro-inflammatory cytokine generated by activated macrophages, performs a crucial role in inflammation and is the main moderator of apoptosis, as reported by ***Gök and Deveci (2022)***.

The coadministration of LGEO and MZ resulted in a partial improvement in the immunohistochemical response of renal tissues to TNF-α and p53. This suggests that LGEO has anti-inflammatory and antiapoptotic properties. These findings are consistent with the defensive impacts of LGEO reported by ***Setiyowati et al. (2022)*** and ***Shalaby et al. (2023)*** against lead acetate-induced reproductive damage and Perfluorooctane Sulfonate-induced jejunal damage, respectively. In both studies, LGEO suppressed pro-inflammatory cytokines like TNF-α and downregulated caspase-3, confirming its antiapoptotic effects.

CONCLUSION

In summary, MZ has toxic renal effects in rats which are characterized by elevation in kidney functions parameters, as well as noticeable alterations in the histopathological and immunohistochemical properties of the organ. The mechanism of MZ-induced toxicity occurs through the induction of oxidative stress, inflammation, DNA damage, and apoptosis in rat renal tissue. The LGEO improved these changes to some extent.

RECOMMENDATIONS

Based on the results of the experiment, the following recommendations are suggested: raising awareness among the farmers and workers about the probable MZ health risks and ensuring the appropriate usage and safety measures when handling it to reduce exposure risk; exploring the use of LGEO as a prophylactic measure to counteract the toxic effects of MZ; conducting further research on the potential benefits of essential oils as antioxidants and promoters of good health.

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