GreenSynthesis of Manganese Oxide Nanoparticles Using Basil Extract for Biocompatibility and Therapeutic Targeting of Metribuzin Poisoned Heart and Lung Tissues in *Wistar* Rats

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Abstract

Manganese oxide nanoparticles have garnered interest for use in a variety of fields, such as biomedical applications, including cancer theranostics and drug delivery. This work aimed to investigate the potential therapeutic and preventative benefits of green-produced MnO NPs made from basil extract against metribuzin-induced oxidative stress, metabolic toxicity, inflammation, and histological changes in the lungs and heart. Green synthesis of MnO NPs using basil extract was done. The shape and size distribution of the MnO nanoparticles were analyzed using TEM and SEM. 18 male albino Wistar rats were divided into three groups (n = 6), which consisted of a control group,

a metribuzin-treated group, and a MnO NPs-treated group. The objective of this study was to determine the increase in mass for each organ and to evaluate the oxidative stress status by measuring the levels of SOD, GPx, GSTs, CAT, GSH, and MDA in the lungs and heart tissues. Additionally, the tissue histology of the organs was examined, and various biochemical parameters (GOT, LDH, and CPK) and inflammation markers (WBC, Lymph, Mid, and Gran) were estimated.The green synthesis of MnO NPs is shown by the gradual shift in color from golden yellow to dark brown. . The morphological characteristics and particle size distribution of the MnO NPs were identified using TEM and SEM. The analyses revealed that the MnO NPs were uniformly spherical in shape, with an average particle size of 6.52 ± 0.88 nm. Results of the *in vivo* rats' study showed that treatment with metribuzin induced growth in the weight of organs, oxidative stress, biochemical toxicity, inflammation, and histological changes in the lungs and heart, as well as a significant amelioration of MnO NPs against the toxic effects induced by metribuzin by reversing all of the previous parameters. In conclusion, the results of the *in vivo* investigation showed that rats given metribuzin herbicide suffered from organs weight gain, oxidative stress, biochemical toxicity, inflammation, and histological alterations in their lungs and heart. Additionally, MnO NPs show effective therapeutic and preventive actions against lungs and heart damage caused by metribuzin. Basil extract's phytochemical components enhance MnO NP biocompatibility, reduce toxicity, and provide antioxidant and anti-inflammatory properties, making them safe and therapeutic for biomedical applications.

Keywords: MnO NPs; Metribuzin; Oxidative stress; Inflammation; Lungs; Heart.

1. Introduction

Metribuzin, identified as a 4-amino-6-(1,1-dimethyl)-3-(methylthio)-1,2,4-triazin 5(4H)-one compound categorized as an asymmetric triazine, was officially approved for use in the United States in 1973 specifically for herbicidal utilization (1). Metribuzin is frequently utilized in the cultivation of potatoes, soybeans, peas, tomatoes, and lentils (2). Adverse impacts on humans, fish, and domestic animals have been recorded in relation to its usage (3). Metribuzin, a xenobiotic, explicates the mechanisms of toxicity in the majority of xenobiotics, which are chemical compounds that are

foreign to the body and include herbicides and environmental pollutants. These mechanisms include disturbance of the body's overall antioxidant capacity and stimulation of free radical production-induced lipid peroxidation (4).

Over the last several years, manganese oxide nanoparticles have garnered interest for use in a variety of fields, such as water treatment, catalysis, and solar cells, as well as in biomedical applications, including biosensors and bioimaging, cancer theranostics, and drug delivery (5). Manganese oxides are a mixed oxide substance that finds extensive application in fields such as electrochemistry, medicine, and catalysis. This is because they are affordable, environmentally benign, occur in diverse forms of manganese, and are abundant in nature (6). Nanoparticles can be created using a variety of methods, such as chemical, physical, and biological ones (7). Green synthesis of nanoparticles has been developed to lower costs, minimize pollution, improve the environment, and protect human health by using plant extracts instead of industrial chemical components to reduce metal ions (8). Phytochemicals found in medicinal plants can be used to create biocompatible, affordable, and renewable green nanoparticles (9). The purpose of the current study was to examine the therapeutic and preventive effects of MnO NPs greenly produced using basil extract against the growth in weight of organs, oxidative stress, biochemical toxicity, inflammation, and histological alterations caused by metribuzin exposure in the lungs and heart.

2. Materials and Methods:

2.1.Plant Materials Collection

Professor Youssef Hallis identified the plant used in this experiment. The basil was harvested in August 2022 from the El-Oued (Guemar) region of Algeria. The basil leaves were cleaned, dried, and stored at room temperature away from direct sunlight. A mechanical grinder was then used to grind the dry leaves into a fine powder. *Ocimum basilicum* L. powder is stored at room temperature in airtight containers until the experiment starts.

2.2.*Aqueous Extract Preparation*

Ten g of dried leaves were boiled for two hours at 50 °C in 100 ml of distilled water to make basil aqueous extract. The extract was macerated for 24 hours at room temperature then filtered through Whatman filter paper. Following that, a rotary evaporator was used to evaporate it, and an oven was used to dry it.

2.3.Compounds Analysis

The aqueous plant extract was subjected to a thorough examination using a standardized methodology to identify the presence of different phytochemicals.

2.4. Green Synthesis of MnO NPs

Manganese oxide nanoparticles were created using a green synthesis approach mediated by *Ocimum basilicum* L. leaf extract, but with minor changes based on the methodology of Saod *et al*. (10). This procedure entailed the amalgamation of the aqueous extract of basil with a manganese (II) chloride ($MnCl_2.4H_2O$) solution. To attain a pH of 8, sodium hydroxide (NaOH) solution was incrementally added to the mixture while maintaining continuous stirring, which facilitated the generation of diminutive particles. The resultant solution was subsequently subjected to magnetic stirring at a temperature of 65 °C for a duration of 6 hours, during which a colorimetric transition from golden yellow to dark brown occurred, indicative of the successful biosynthesis of MnO NPs. Following this, the mixture was centrifuged at 5000 rpm for 20 minutes, after which the supernatant was discarded. The resulting precipitate was subjected to three washing cycles utilizing distilled water and ethanol prior to being desiccated to yield the final product.

2.5. Characterization of MnO NPs

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses were conducted to evaluate the morphological characteristics and particle size distribution of the manganese oxide nanoparticles (MnO NPs) greenly synthesized using basil extract.

.2.6.Animal Care and Experimental Design:

In our investigation, 18 male Wistar rats, weighing 206±9.02 g, were used. The rats were 08 weeks old when they were obtained from the Pasteur Institute in Algiers. The Faculty of Natural and Life Sciences at Echahid University Hamma Lakhdar-El-Oued's animal husbandry laboratory is where these animals were grown. The rats had the same living conditions, including a 12-hour photoperiod of light and darkness and room temperature, and were kept in plastic cages with metal mesh coverings. They were also given a standard diet and free access to food and water. Over the course of 08 weeks, the experiment was conducted.

Following a two-week adaptation period, the animals were divided up into 03 experimental groups, each with six animals:

Group 1 (Control): Healthy rats received water and administered intraperitoneally with physiological saline solution.

Group 2 (MTZ): Rats exposed to metribuzin and administered intraperitoneally with physiological saline solution.

Group 3 (MnO NPs): Rats exposed to metribuzin and administered intraperitoneally with MnO NPs (one dose/day, 05 mg/kg).

Oral dose of metribuzin in drinking water (220 mg/kg) was used to cause intoxication for eight weeks. The rats received four weeks of treatment with MnO NPs. We injected groups 1 and 2 with physiological saline solution to subject rats to the same experimental conditions.

2.7. Sacrifice, Blood Sampling and Tissues Collection

Following an 8-week treatment period and a 16-hour fast, the rats were sacrificed by inhaling a small amount of chloroform (94%). During the animal sacrifice process, blood samples have been placed into dry tubes to obtain serum by centrifugation for 10 minutes at 3000 tour/min, which was used to assess GOT, LDH, and CPK activity, and into EDTA tubes for leukocyte line studies. the lungs and heart were carefully sampled, cleaned, weighted, and kept at -20°C in order to homogenates preparation to measure oxidative stress. In addition, the organs were preserved in 10% formaldehyde for histological examination.

2.8. Biochemical Parameters and Inflammation Markers

The following reference codes, which correspond to commercial kits from Spinreact, represent the enzymatic activity of aminotransferase (GOT), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK): GOT-1001161, LDH-1001260, and CPK-1001217. The hematology autoanalyzer (Sysmex) measures the levels of inflammation markers (WBC, Lymph, Mid, and Gran).

2.9. Tissue Samples Preparation and Lungs and Heart Oxidative Stress Parameters

The procedure used by Boulaares *et al.* was followed to prepare homogenates from lungs and heart tissue. Following Beauchamp and Fridovich (11), Flohe and Gunzler (12), Habig *et al*. (13), Regoli and Principato (14), Weckbecker and Cory (15) and Draper and Hadley (16) methods, the levels of SOD, GPx, GSTs, CAT GSH, and MDA levels were measured, respectively.

2.10. Histopathological Study

Following the rats' sacrifice, the tissues from the lungs and heart were removed and kept in a fixative solution containing 10% formaldehyde until it was time to prepare the slides. The tissues were then washed with toluene, immersed in paraffin, and stained with hematoxylin and eosin after being dehydrated using an increasing series of ethanol. The final slides had been examined under a microscope that had a camera attached, and the computer screen showed the pictures that the camera had taken.

2.11. Statistical Analysis

In order to express the results as either an average ± ES (standard deviation), the study used the student's t-test for independent samples. Minitab 13.0 software was used to analyze all the data, and a P-value of less than 0.05 was used to assess statistical significance.

3.Results

3.1.Bioactive Compounds Analysis

Phytochemical examination revealed the presence of phenols, flavonoids, catechic tannins, terpenes, saponins, reducing sugars, and alkaloids in *Ocimum basilicum* L. aqueous extract **(Table 01).**

Table 01: Bioactive compounds of *Ocimum basilicum* L. aqueous extract.

Bioactive compounds	Basil extract	Test
Phenols	+	Ferric chloride test
Flavonoids	+	Magnesium test
Catechic tannins	+	Ferric chloride test
Terpenes	+	Salkowki's test
Saponins	+	Froth test
Reducing sugars	+	Fehling test
Alkaloids	+	Dragendorff's test

3.2.Synthesis and Characterization of MnO NPs

The change in color to dark brown from golden yellow over time indicates the green synthesis of MnO NPs through the use of basil extract **(Figure 01)**.

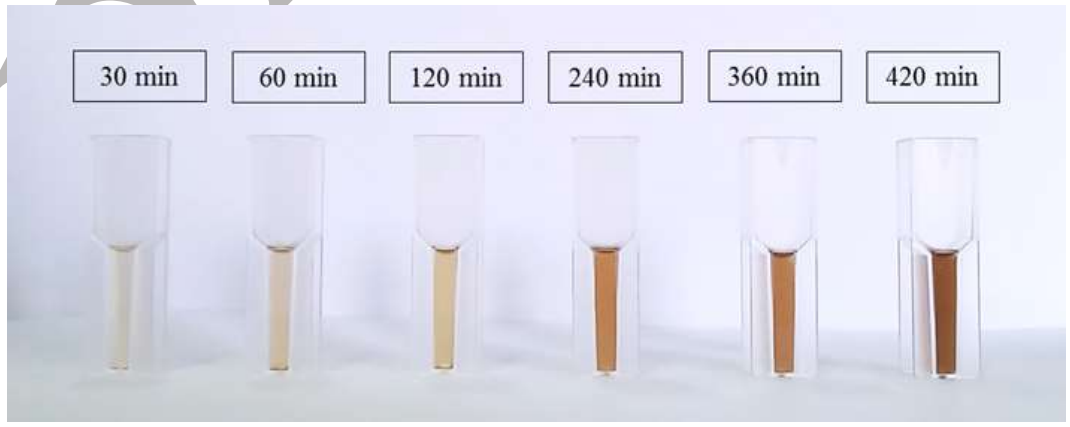

Figure 01: Green synthesis of MnO NPs using *Ocimum basilicum* L. extract at different time.

The images from the scanning and transmission electron microscopes **(Figure 02 A/B)** show that the MnO NPs are uniformly spherical in shape and have a homogeneous dispersity. The MnO NPs' average particle size was determined to be 6.52 ± 0.88 nm **(Figure 02 C)**.

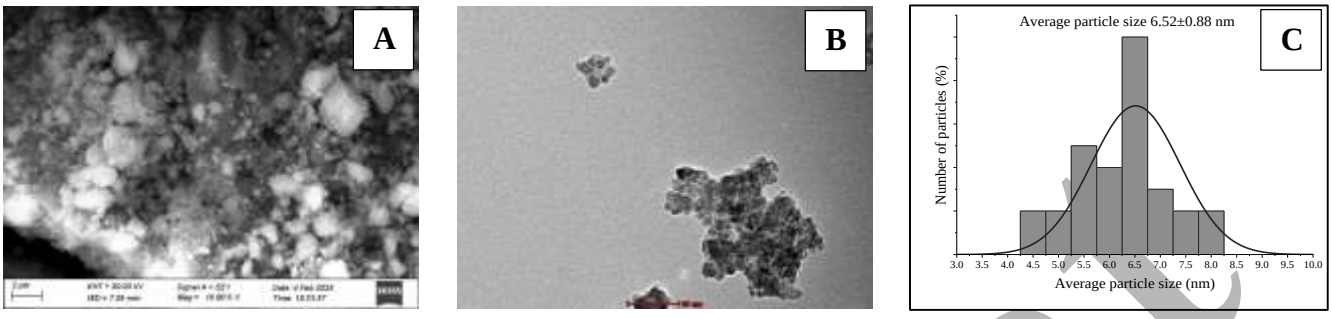

Figure 02: MnO NPs' morphological characteristics based on SEM image (A), TEM image (B) and their diameter (nm) (C).

3.3.Relative Lungs and Heart Weight

The MTZ group showed a significant rise in relative lung and heart weight when compared to the control group, but the MnO NPs group showed a significant decrease when compared to the MTZ group (Table 02).

Table 02: Relative lungs and heart weight in control and MnO NPs group.

	Control	MTZ	MnO NPs
Lungs	0.665±0.005	2.138±0.503**	0.990±0.110 */###
Heart	0.254±0.003	0.324±0.023 *	0.298±0.004 ***/##

Data are expressed as mean ± SD (n=6).

***** p<0.05, ******p<0.01, *******p<0.001: significantly different from control group.

# p<0.05, ##p<0.01, ###p<0.001: significantly different from metribuzin exposed group.

3.4.Biochemical Parameters

The activity levels of GOT, LDH, and CPK showed a significant increase (P<0.05) in the MTZ group in comparison to the control group, and there was also a partial improvement observed in the MnO NPs group when contrasted with the MTZ group **(Figure 03)**.

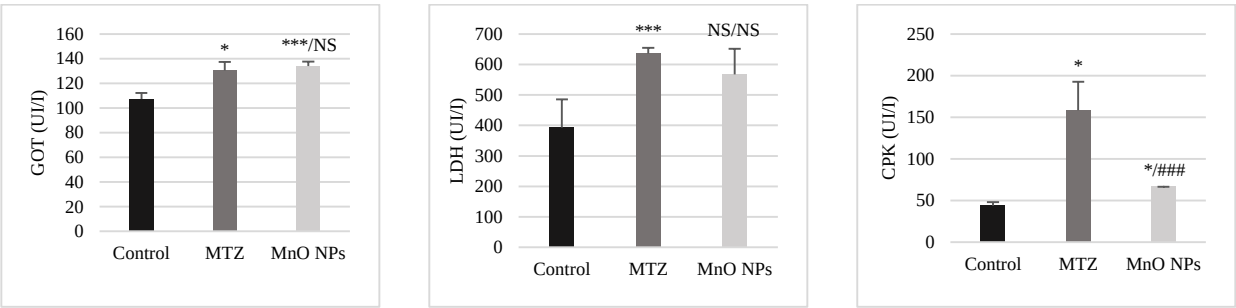

Figure 03: GOT, LDH, and CPK activity in heart in control and MnO NPs group.

3.5.Inflammation Markers

The levels of inflammation markers (WBC, Lymph, Mid, and Gran) in the MTZ group were found to be significantly higher than those in the control group ($P<0.05$). On the other hand, there was a highly significant decrease ($P<0.001$) in the levels of WBC, Mid and Gran with no significant change ($P>0.05$) in the levels of Lymph in the MnO NPs group compared to the MTZ group **(Figure 04)**.

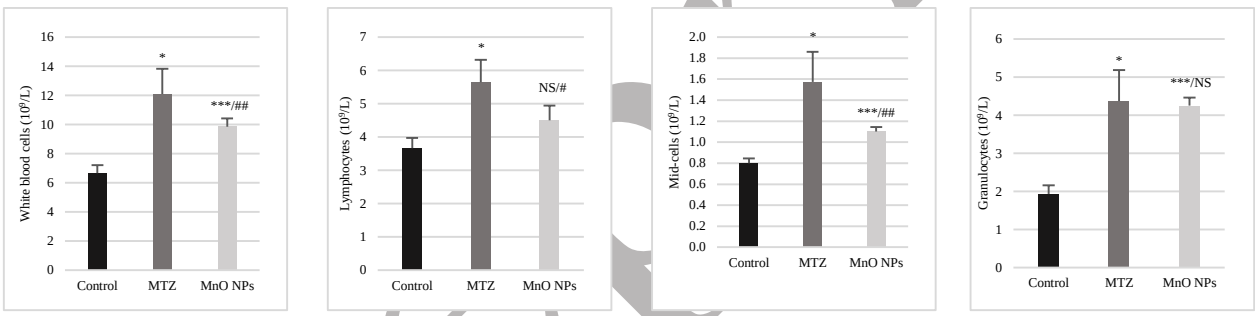

Figure 04: Inflammation markers level in control and MnO NPs group.

3.6.Oxidative Stress Parameters

The results of the enzymatic activity tests indicated that there were no noticeable changes in the activity of SOD and CAT in the lungs, a significant increase in the activity of GSTs in both the lungs and heart, and a significant decrease in the activity of GPx in the lungs as well as in the activity of SOD, GPx, and CAT in the MTZ group compared to the control group. In contrast, there was only a partial improvement in the activity of these enzymes in the group treated with manganese oxide nanoparticles (MnO NPs) compared to the MTZ group **(Figure 05)**.

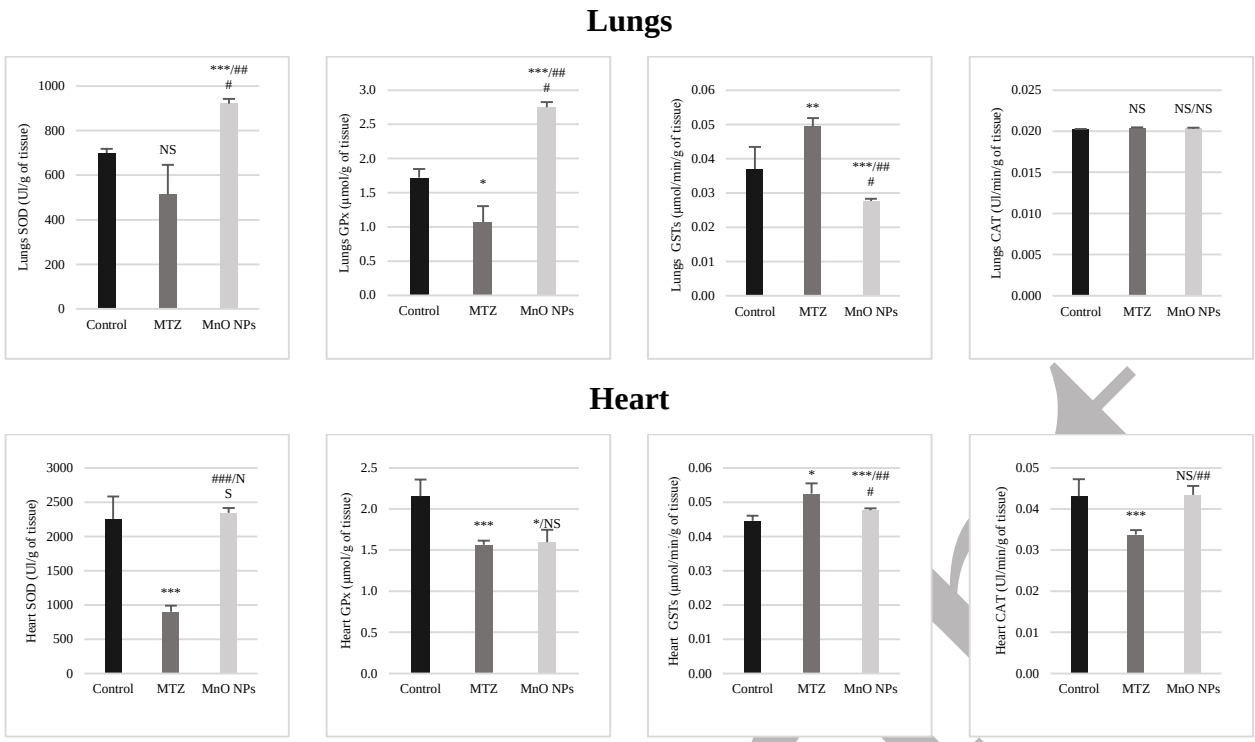

Figure 05: Enzymatic antioxidant activity in lungs and heart in control and MnO NPs group.

Our research findings showed a significant decrease in the level of GSH in both the lungs (P<0.01) and heart (P<0.001) tissues of the MTZ group compared to the control group. Additionally, there was a very highly significant increase in MDA level in both tissues (P<0.001). However, there was a remarkable improvement in GSH and MDA levels in the MnO NPs group versus the MTZ group **(Table 03)**.

Table 03: GSH and MDA levels in lungs and heart in control and MnO NPs group.

	Control	**MTZ**	**MnO NPs**
		Lungs	
GSH levels (nmol/g of tissue)	9.377±0.606	4.402±0.897 **	7.946±0.36 */###
MDA levels (nmol/g of tissue)	8.627±0.305	10.088±0.205 ***	9.647±0.105 ***/##
		Heart	
GSH levels (nmol/g of tissue)	3.8713±0.0778	2.6428±0.0553 ***	3.058±0.311 */ NS
MDA levels (nmol/g of tissue)	17.460±1.64	25.150±0.913 ***	21.920±1.15 */#

Data are expressed as mean ± SD (n=6).

***** $p<0.05$, ******$p<0.01$, *******$p<0.001$: significantly different from control group.
$^{\#}$ $p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$: significantly different from metribuzin exposed group.

3.7.Histopathological Study

The control rat's lung section showed typical histological features, including thin alveolar septa. In contrast, the MTZ-treated group's lung tissue exhibited several histological changes, such as thickened interalveolar septa and completely blocked air spaces. These areas displayed evidence of hemorrhage and inflammatory cell infiltration within the markedly thickened septa. The MnO NPs group demonstrated partial lung tissue recovery, with some alveoli remaining collapsed while others were expanded and ruptured. Control rat heart tissues revealed normal myofibrillar structure and cells under microscopic examination. Conversely, MTZ-treated rat heart tissues showed muscle fiber abnormalities, including hemorrhage, inflammation, necrosis, and vacuolization of cardiomyocytes. The MnO NPs group, however, exhibited considerable improvement, with heart tissue morphology more closely resembling that of the control group **(Figure 06/Table 04).**

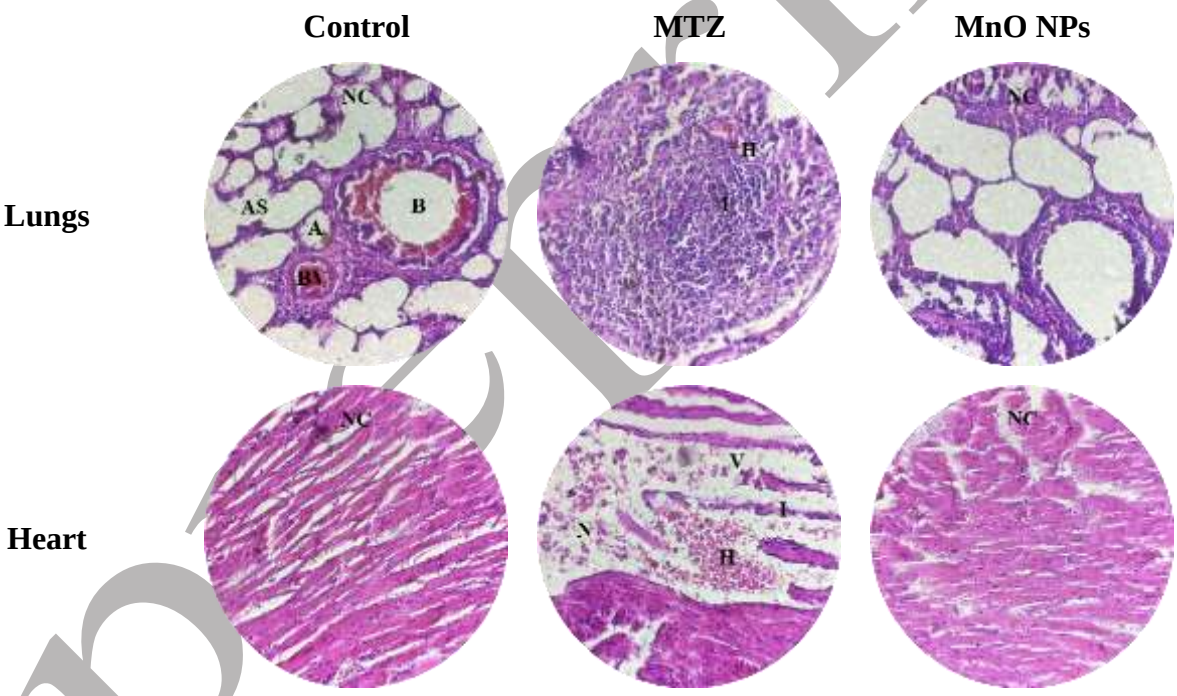

Figure 06: Photomicrographs of lungs and heart section of all experimental groups, stained using hematoxylin and eosin (H&E), shown at 40 x 10 magnification.

Normal cells: **NC**; Hemorrhage: **H**; Inflammation: **I**; Vacuolization: **V**; Necrosis: **N**
Bronchiole: **B**; Alveolus: **A**; Blood vessels: **BV**; Alveolar sacs: **AS**

Table 04: Grading of histological alterations in the lungs and heart sections of all experimental groups.

Parameters	Control	MTZ	MnO NPs

Hemorrhage	**Lungs**	-	++	-
	Heart	-	+++	-
Inflammation	**Lungs**	-	+++	-
	Heart	-	++	-
Vacuolization	**Lungs**	-	-	-
	Heart	-	+++	-
Necrosis	**Lungs**	-	-	-
	Heart	-	+++	-

None (-); Moderate (+); Severe (++); Very severe (+++)

4. Discussion

This study investigated the therapeutic and preventive benefits of MnO NPs against metribuzin-induced organs weight gain, oxidative stress, biochemical toxicity, inflammation, and histological alterations in the heart and lungs.

Our study revealed the presence of phenols, flavonoids, catechic tannins, saponins, reducing sugars, alkaloids, and terpenes in basil aqueous extract. Based on the information from **Nadeem *et al.*** (17), the current findings are consistent with their results, which indicated the presence of alkaloids, phenols, tannins, flavonoids, terpenoids, steroids, and glycosides in the basil leaf aqueous extract. According to published research, the active substances in medicinal plants called phytochemicals are thought to have pharmacological potential.

The solution's hue changes from golden yellow to dark brown in relation to the green synthesis of MnO NPs, indicating the biogenesis of MnO NPs utilizing basil extract. Initially, the optical observation of color modification was used to confirm the plant extract's ability to create nanoparticles (18).

The color shift of the metal ion solution, which serves as a visual cue, is necessary for the synthesis of MnO NPs, as **Khan *et al.*** confirmed (19). The average particle size and shape were investigated using TEM and SEM pictures. The findings showed that the average size of MnO NPs was 6.52 ± 0.88 nm and that they were spherical in shape.

Comparing the MTZ group to the control group, there was a significant increase in relative lung and heart weight; whereas, there was a significant decrease in the MnO NPs group when compared to the MTZ group .Changes in the weight of the organs, either in absolute terms or in relation to body weight, are extremely sensitive markers of early toxicity, especially when under strictly regulated circumstances, like in an experiment (20). Experimental rats who received subacute metribuzin may have experienced negative effects, as evidenced by the significant increase in the absolute and relative weight of several vital organs (3). It has been demonstrated that plant extract-mediated

synthesis can decrease toxicity while improving the biological properties (bioavailability, biocompatibility, cell internalization, and antioxidant activity) of metal and metal oxide nanoparticles (5). Because MnO NPs are less harmful, they have become more important in the synthesis and production processes, which eliminates the metribuzin effect responsible for lungs and heart hypertrophy.

When comparing the MTZ group to the control group, the activity levels of GOT, LDH, and CPK significantly increased, and the MnO NPs group showed partial improvement in comparison to the MTZ group. The three primary cardiac enzymes that are measured are GOT, LDH, and CPK. The enzymes present in cardiomyocytes can be found in the blood, and when cardiac cells suffer inflammation (myocarditis) or necrosis (myocardial infarction) due to a variety of reasons, the activity (content) of these enzymes increases (21). The outcomes observed indicate that manganese oxide nanoparticles could potentially have cardioprotective effects by regulating metribuzin-induced oxidative stress and inflammation, which are the principal causes of cell damage and the release of their contents into the blood.

In comparison to the control group, the MTZ group exhibited noticeably higher levels of inflammatory markers. On the other hand, the therapy group showed a significant decrease in inflammatory marker levels as compared to the MTZ group. One important organ to focus on when exposed to pesticides is the immune system (22). The capacity of pesticides to either promote or inhibit lymphocyte proliferation and cytokine synthesis, in addition to inducing genetic injury and chromosomal irregularities in cultured lymphocytes, has been proposed as plausible mechanisms underlying their adverse effects on the immune system (22). The large surface area to volume ratio of these entities allows them to possess high surface reactivity, which in turn facilitates their interaction with biological membranes and promotes their physical transport within the membrane (10). Nanoparticles exhibit improved penetration in epithelial and inflammatory cells, which contributes to their superior effectiveness and longer persistence in treatment. Additionally, they exhibit better targeting of specific sites, such as inflammatory cells or tissues (23). Inhibiting pro-inflammatory cytokines is a crucial process, as these cytokines enhance immune responses and are targeted by the vast majority of nanoparticles (23).

The evaluation of oxidative stress biomarkers (SOD, GPx, GSTs, CAT, GSH, and MDA) in the pulmonary and cardiac tissues of male rats revealed that MTZ induces oxidative stress. Concurrently, a significant amelioration was observed in the MnO NPs group in contrast to those in the MTZ group. The antioxidant defense mechanisms, including glutathione (GSH), catalase (CAT), glutathione peroxidase (GST), and superoxide dismutase (SOD), can be inhibited by xenobiotics, which cause the overproduction of untimely and excessive free radicals. This leads to the damage of macromolecules, including DNA (4). Certain pesticides have the potential to induce a rise in reactive

oxygen species (ROS) generation, thus resulting in oxidative stress in unintended organisms (3). Metal oxide nanoparticles (NPs), specifically manganese dioxide, can effectively replicate the functions of antioxidant enzymes by catalyzing the breakdown of superoxide anions and hydrogen peroxide (7).

The observed increase in organ mass, oxidative stress levels, biochemical toxicity, and inflammatory responses documented in the present investigation were substantiated through histopathological analyses of pulmonary and cardiac tissues. Control rats displayed normal histological features in their pulmonary and cardiac tissues upon examination. In contrast, the MTZ-treated group's lungs and heart samples showed various structural changes. Interestingly, the MnO NPs group exhibited considerable enhancement, with their lung tissue structure closely resembling that of the control group. There exists indirect corroborative evidence that associates pesticide exposure with specific chronic health conditions, including respiratory ailments, notably chronic obstructive pulmonary disease, as well as cardiovascular disorders (2). Research involving animal models has indicated that metribuzin may induce deleterious health consequences, such as alterations in tissue histology (4). Moreover, individual pesticides have been documented to elicit cellular toxicity via oxidant-mediated mechanisms, which encompass both programmed and unprogrammed cell death, lipid membrane damage, metabolic disruption, modification of diverse signaling pathways, or alteration of tight junction integrity (24). This elucidates the varied histological alterations detected in the tissue specimens from the MTZ-exposed group. The amelioration observed in all aforementioned parameters is substantiated by the findings derived from the histological evaluations of the tissue sections. The inverse of the aberrant ROS generation process, MnO_2 NPs can consume excess H_2O_2 in situ and convert it to O_2. Additionally, MnO_2 particles can control the degree of inflammation by affecting the expression of genes that produce cytokines. MnO_2 gradually breaks down during this process to produce Mn^{2+}, which is expelled with bodily fluids and aids in the return of the body's internal environment to its ideal state (25). We suggest that the phytochemical components of the basil extract used in the green synthesis of MnO NPs play a crucial role in enhancing their biocompatibility by acting as natural reducing, capping, and stabilizing agents, which help produce uniformly sized and shaped nanoparticles. These plant-derived compounds reduce toxicity by eliminating the need for harsh chemicals and create a bio-friendly surface that improves cellular interactions and uptake. Additionally, functional groups from phytochemicals enhance biological compatibility, while their antioxidant and anti-inflammatory properties further contribute to the safety and therapeutic value of MnO NPs, making them well-suited for biomedical applications.

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Authors' Contribution

Study concept and design: I.B.

Acquisition of data: I.B., S.C.

Analysis and interpretation of data: I.B., S.D.

Drafting of the manuscript: I.B.

Critical revision of the manuscript for important intellectual content: S.D., J.N.

Statistical analysis: I.B., S.D.

Administrative, technical, and material support: I.B., S.C.

Study supervision: S.D., J.N.

All authors have read and agreed to publish version of the manuscript.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare no competing interests.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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