



The Palliative Effects of *Moringa Oleifera* – Mediated Iron Oxide Nanoparticles Against Mercury – Induced Immune Responses and Mmp9 mRNA Expression in Rats.

Research Article

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DOI: <https://doi.org/10.5281/zenodo.16786958>

ABSTRACT

This study evaluated the palliative effects of *Moringa oleifera*-mediated iron oxide nanoparticles (MOLE-FeNPs) against mercury-induced immune responses and Mmp-9 mRNA expression in rats. *Moringa oleifera* leaves were extracted and utilized in the green synthesis of iron oxide nanoparticles, which were characterized using UV-Vis, SEM, EDX, XRD, and FTIR analyses. Forty male rats were divided into eight groups each receiving combinations of MOLE, FeNPs, MOLE-FeNPs, and mercury for 28 days. Mercury exposure significantly elevated inflammatory cytokines (INF- γ , IL-1 β , TNF- α , IL-6, IL-10, TGF- β 1, NF- κ B, IgM, IGF-1) and Mmp-9 mRNA expression. Treatment with MOLE, FeNPs, and MOLE-FeNPs ameliorated these effects, with MOLE-FeNPs showing the most pronounced reversal, restoring parameters toward control levels. The green-synthesized FeNPs demonstrated potent immunomodulatory properties, indicating their potential as a protective supplement against mercury-induced immune deregulation and apoptosis related gene expression.

Keywords: Palliative, Nanoparticles, Mercury, Response.

1 Introduction

The science of Nanotechnology deals with the creation, investigation and utilization of systems that are 1000 times smaller than the components currently used in the field of microelectronics. Nanotechnology has attracted extensive worldwide attention with potential applications in many fields, including medical, photocatalytic, food packaging and electronics sectors (Basnet et al., 2018; Shirsat et al., 2015).

About 40% of the population reported using medicinal plants for therapeutic purposes, and the usage of herbal medicines is increasing (Ekor, 2014). The *Moringa oleifera* (MO), a member of the Moringaceae family, is known as "The Miracle Tree" and is grown in many nations throughout the world (Bhattacharya et al., 2018). It has been utilized for millennia due to its beneficial nutritional and health-promoting features around the world (Vergara-Jimenez et al., 2017). The medicinal properties of MO include hepatoprotective, neuroprotective, antioxidant, anti-inflammatory, anti-carcinogenic, antimicrobial, and immune-improving activities (Abd-Elhakim et al., 2018; El-Hadary and Ramadan, 2019; El Shanawany et al., 2019; Obembe and Raji, 2018). MO also produce significant amounts of saponins, tannins, flavonoids, glycosides,

Mansour et al., (2014) showed that MO has renal protective benefits against heavy metal poisoning by controlling renal functions, preventing oxidative damage, and suppressing inflammatory responses. A variety of chronic disorders are also avoided or treated with MO. Abou-Zeid et al., (2020), Khalil et al., (2020), and Mansour et al., (2020) all reported on the effectiveness of MOLE against tilmicosin-induced nephrotoxicity, cardiac toxicity in rats, and reproductive



toxicity. Anwar et al., (2007) conducted a short study on the antibacterial, anti-inflammatory, anti-cancer, and anti-diabetic properties of moringa oleifera leaves (MOL).

According to Karthivashan et al., (2015), MOL includes minerals, vitamins, carotenoids, phenolic acids, and flavonoids that contribute to the antioxidant benefits of MOL. The polyphenol content of MOLE has a potent anti-oxidant activity against free radicals and lessens the effects of oxidative stress (OS) (Sreelatha and Padma, 2009). Aja et al., (2014) came to the conclusion that methanolic MOLE has chemical components that are absent from MO seeds.

The evolvement of green and sustainable methods for nanoparticles synthesis has been a recent research focus (Adil et al., 2015). Frequently, plant extracts are effective precursors for the production of metallic nanoparticles (Khan et al., 2018). Iron nanoparticles (FeNPs) are sub-micrometer particles of iron metal. They are highly reactive because of their large surface area. In the presence of oxygen and water, they rapidly oxidize to form free iron ions and they are non-toxic. FeNPs, are frequently synthesized using various plants, such as green tea (Shahwan et al., 2011), Sorghun sp (hybrid sorghum) (Njagi et al., 2011), Eucalyptus globulus (Madhavi et al., 2013), Pomegranate (Rao et al., 2013), plantain peel (Venkates warlu et al., 2013), Dodonaea viscosa (Kiruba Daniel et al., 2013), Tridax procumbens (Senthil and Ramesh, 2012), Grape seed proanthocyanidin (Narayanan et al., 2012).

Mercury is a heavy, silvery-white metal that is liquid at room temperature. Compared to other metals, it is a poor conductor of heat, but a fair conductor of electricity. (Hammond, 2008) Mercury and most of its compounds are extremely toxic and must be handled with care. Toxic effects include damage to the brain, kidneys and lungs. Mercury poisoning can result in several diseases, including acrodynia (pink disease), Hunter-Russell syndrome, and Minamata disease.

This present study deals with the palliative effect of Moringa oleifera-mediated iron oxide nanoparticles against mercury-induced immune responses and Mmp 9, mRNA expression in rats. This study involves the preparation of Moringa oleifera leaf extract, the synthesis and characterization of green iron oxide nanoparticles using moringa oleifera leaf extract and the analysis of the toxic effect of mercury on cellular functions, the use of Moringa oleifera as reducing agent to make iron-based bio-friendly nanoparticles, and ameliorative effect of green iron-based nanoparticle on the expression of Matrix metalloproteinase 9 (Mmp-9) mRNA gene induced by mercury.

2 Literature Review

Cross cross-sectional survey design was used for the study. The data were collected from the respondents at a single point for the period of the study.

Moringa oleifera is a fast-growing, drought-resistant tree of the family Moringaceae, native to the Indian subcontinent. *M. oleifera* is a fast-growing, deciduous tree that can reach a height of 10–12 metres (33–39 feet) and trunk diameter of 45 centimetres (18 inches) (Parotta and John, 1993). *Moringa oleifera* produce large quantities of saponins, tannins, flavonoids, glycosides, terpenoids, beta-carotene, vitamin C, vitamin E, and polyphenols, all of which display medicinal properties, including, renal protective, hepatoprotective, neuro protective, antioxidant, anti-inflammatory, anti-carcinogenicity, anti-microbial and immune-improving activities (Obembe and Roji, 2018; Zeng et al., 2019).

M. oleifera has numerous applications in cooking throughout its regional distribution. Edible parts of the plant include the whole leaves (leaflets, stalks and stems); the immature, green fruits or seed pods; the fragrant flowers; and the young seeds and roots (Lim, 2012). Ground, debittered moringa seed is suitable as a fortification ingredient to increase the protein, iron and calcium content of wheat flours (Oyeyinka, and Oyeyinka, 2018). *Moringa oleifera*, is classified as an important herbal plant due to its immense medicinal and non-medicinal benefits. Traditionally, the plant is used to cure wounds, pain, ulcers, liver disease, heart disease, cancer, and inflammation. The pharmacological studies confirm the hepatoprotective, cardioprotective, and anti-inflammatory potential of the extracts from the various plant parts. It was found that bioactive constituents are present in every part of the plant (Pareek et al., 2023). These properties of *Moringa oleifera*, makes it an ideal candidate for green synthesis of nanoparticles.

Nanotechnology has emerged as a state-of-the-art and cutting-edge technology with multifarious applications in a wide array of fields. Natural products or extracted from natural products, such as different plant extracts, have been used as



reductants and as capping agents during synthesis. A very easy, efficient, and environment-friendly protocol was developed to synthesize green nanoparticles (NPs) with an aqueous extract of various plant, phenolic compounds extracted from plants play a major role as non-toxic reducing and capping agents for nanoparticles. Nanoparticles and their compounds are known to exert a strong inhibitory and microbial activity on bacteria, viruses, and fungi (Abderrhmane and Salah, 2021).

Green Synthesis: The evolvement of green and sustainable methods for nanoparticles synthesis has been a recent research focus (Adil et al., 2015). Frequently, plant extracts are effective precursors for the production of metallic nanoparticles (Khan et al., 2018). Iron nanoparticles (FeNPs) are sub-micrometer particles of iron metal. They are highly reactive because of their large surface area. In the presence of oxygen and water, they rapidly oxidize to form free iron ions and they are non-toxic. FeNPs, are frequently synthesized using various plants, such as green tea (Shahwan et al., 2011), Sorghum sp (hybrid sorghum) (Njagi et al., 2011), Eucalyptus globulus (Madhavi et al., 2013), plantain peel (Venkates warlu et al., 2013), Dodonaea viscosa (Kiruba Daniel et al., 2013), Tridax procumbens Grape seed proanthocyanidin (Narayanan et al., 2012). Iron oxide nanoparticles (Fe₃O₄ NPs) synthesized using *M. oleifera* extracts are biocompatible, eco-friendly, and cost-effective. Plant metabolites act as reducing and stabilizing agents, influencing nanoparticle size, shape, and biological activity (Kiwumulo et al., 2022).

2.1 Iron Oxide Nanoparticles and Biomedical Applications

Iron oxide nanoparticles have shown promise in various biomedical fields due to their magnetic properties, low toxicity, and ability to cross biological barriers. Its application in drug delivery, antimicrobial activity, tissue engineering, and detoxification is due to its size and shape. The smaller, spherical nanoparticles exhibit better cellular uptake and therapeutic efficacy (Meng et al., 2024).

2.2 Mercury

Mercury is a heavy, silvery-white metal that is liquid at room temperature. Compared to other metals, it is a poor conductor of heat, but a fair conductor of electricity. (Hammond, 2008) Mercury and most of its compounds are extremely toxic and must be handled with care. Mercury is widely used in medicine, agriculture, and industry. Meanwhile, according to the World Health Organization, it has been ranked as one of the ten most hazardous substances in the world, with the Agency for Toxic Substances and Disease Registry ranking it third. It has no known positive functionality in the human body, and even at low concentrations, it can have harmful long-term health effects, seriously affecting the healthcare system as well as posing a serious public health threat. It can cause disorders such as various cancers; endothelial dysfunction; gastric and vascular disorders; liver, kidney, and brain damage; hormonal imbalances, miscarriages, and reproductive disorders; skin lesions; vision damage; and even death. The constant controlling and monitoring of mercury use is a serious public health problem, requiring urgent attention and attentiveness from the governments of all countries and, in the long run, a rapid and concerted response (Charkiewicz et al., 2025).

Mercury exposure induces oxidative stress, inflammation, and immune dysregulation in mammals. The Immune Effects include, disrupts cytokine balance, activates macrophages, and alters lymphocyte function. The Oxidative Stress of mercury Leads to increased reactive oxygen species (ROS), damaging cellular components (Pollard et al., 2019).

The effect of green synthesized metal nanoparticles on heavy metal like mercury, cadmium and so has not been fully elucidated. Its effects in the immune response in human will be guard in handling of heavy metal like mercury since it finds its way to daily applications.

This present study deals with the palliative effect of *Moringa oleifera*-mediated iron oxide nanoparticles against mercury-induced immune responses and Mmp 9, mRNA expression in rats.



3 Materials and Methods

3.1 Chemicals

Essays were done using standard ELISA kits. All other reagents used, unless otherwise indicated, were of analytical grade. Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and sodium hydroxide (NaOH) pellets were purchased from Nigeria. The chemicals were further used without any purification. During plant extraction and nanoparticle synthesis, deionized water was used.

3.2 Plant extract preparation *Moringa oleifera* leaves extract (MOLE)

Moringa oleifera leaves (MOL) were obtained from Ado-Ekiti and authenticated by a Botanist. Dried *M. oleifera* leaves were crushed into a powder using a high-speed milling device. One thousand grams of powder was extracted in ratio 1:1 absolute ethanol/ deionized water for 48 hours then filtered twice through filter paper with 2 μm pore size. The resultant extract was concentrated and evaporated to dryness using a rotary evaporator at 40–45 °C. The residual yield of MOLE extract was 63.8 g/1000 g of dried powder. The obtained extract was kept at 4°C until use.

3.2.1 Synthesis of MOLE based Iron Nanoparticle (MOLE-FeO-NPs)

The green synthesis of iron oxide (Fe_2O_3) nanoparticles using plant extract was performed by a bottom-up process approach and reduction technique by a single pot system revamping the procedure by Chatterjee et al. (2021). At first, the measured amount (100 mL of 3 mM) of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was added to a beaker. Then the plant extract was added slowly into the solution. Finally, the FeCl_3 solution reacted with 100 ml of filtered plant extract. The entire reaction was done at pH 11 by adding 1 M NaOH . The mixture was stirred continuously for a minimum of 1 h and the formation of a dark brown colour solution confirmed the synthesis of MOLE-FeNPs. The synthesized FeO-NPs were separated by centrifugation at 12,000 \times g for 15 min and the pellet containing FeO-NPs were washed (3 times) with deionized water followed by drying at 80 °C for 3 h and stored for further use.

3.2.2 Synthesis of Iron Nanoparticle (FeNPs)

We added 0.1M Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) to 0.2M Iron (IV) sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and stirred. Ammonium Hydroxide solution was added dropwise until colour change to dark brown solution to confirm the synthesis of FeO-NPs.

3.2.3 Characterization of MOLE-FeNPs

The UV–visible spectra of the synthesized FeO-NPs had been recorded using a Mobi Microplate spectrophotometer. The morphological, topographical, size and purity of the nanoparticles were analysed by a scanning electron microscope (SEM) and Energy Dispersive X-ray Spectroscopy (EDX), respectively. The zeta potential (Charge distribution) of the nanoparticles was studied using XRD. To identify the functional groups, present in synthesized FeO-NPs, Fourier transform infrared spectroscopy (FTIR, Shimadzu 8400 S) was employed.

3.4 Experimental Animals

Forty male rats of comparable weight and age (10 ± 2 weeks, $200 \pm 20\text{g}$) housed in standard well-ventilated cages (maximum of 5 animals/cage), in the Animal Holdings of the Department of Science Technology, The Federal Polytechnic Ado-Ekiti, Nigeria. The rats were provided with standard chow and water ad libitum and subjected to the natural photoperiod of 12 h light/dark cycle with room temperature $25 \pm 2^\circ\text{C}$ and humidity $60 \pm 5\%$. All animals were cared for humanely according to the conditions stated in the Guide for the Care and Use of Laboratory Animals of the National Academy of Science (NAS), published by the National Institute of Health. Gavage was given day after day for 28 days, using a feeding needle. All animals were carefully observed throughout the experiment for any signs of intoxication or mortalities.



3.4.1 Blood and Tissue Sample Collection

Twenty-four hours after the last treatment, the over-night-fasted rats were weighed and anesthetized with diethyl ether at the end of the dosing period. Two mL (2mL) blood samples were collected from them via cardiac puncture. Blood was collected using a BD Vacutainer PST II Tubes, left for coagulation, then centrifuged at 322 x g for 20 min for serum separation. Sera were preserved at 20 °C until biochemical analysis. Kidney, liver and testis tissues were collected and divided into four portions. Thirty mg (30mg) was washed in cold saline and mix with RNA shield for storage before RNA extraction.

3.4.2 RT-PCR analysis of apoptosis and stress related gene expression of MMP9

RT-PCR analysis of apoptosis and stress related gene expression of MMP9 is analyzed using Ultra Turrax homogenizer in a cold solution of 0.015 M NaHPO₄ buffer and 0.15 M NaCl (1:6 w/v; pH 7.8) for homogenate preparation for biochemical measurements.

3.4.3 Serum and Tissue Mercury Analysis

1mL/mg of serum/tissues were digested with 10 mL analytical grade H₂O₂ (hydrogen peroxide) and HNO₃ (nitric acid) (1:1) in a Teflon beaker. The solution was then made up to 25 mL with distilled water. Hg concentration in samples were analysed with thermal decomposition coupled atomic absorption spectrophotometry (AAS) (Gao et al., 2012; Santos et al., 2017; Santos et al., 2020). All analysis were investigated in triplicate.

3.4.4 Enzyme-linked immunosorbent assay (ELISA) for Inflammatory Cytokines Assessment of TNF- α

Level of TNF- α was determined using a standard ELISA kit (Elabscience Biotechnology Co., Ltd, USA). 100 μ L of the standard and sample was added to each well, and incubated for 90 min at 37 °C. The liquid was then removed. 100 μ L of Biotinylated Detection Ab. Solution was added and incubated for 1 hour at 37 °C.

3.4.5 Inflammatory Cytokines

Renal tissues (IL-1 β , IL-6, NF- κ B) levels were detected in a 96 well microtiter plate by using a commercial ELISA kit (MEIMIAN Biological Co Ltd, China). The optical densities were read at 450 nm on a microplate reader. The concentrations were calculated from a standard curve.

3.4.6 Total RNA Extraction

Total RNA was extracted from 0.1 to 0.2 g of rat kidney tissues by using Total RNA was extracted using Quick-RNA™ Miniprep Kit (Zymo Research) as per manufacturer's protocol. The quality and quantity of the isolated RNA were assessed using the 260/280 nm absorbance ratio (1.8–2.0 indicates a highly pure sample) using NanoDrop 2000c spectrophotometer (USA). Total RNA was used immediately.

3.4.7 Synthesis of cDNA and RT qPCR Quantification

cDNA synthesis was performed using the ProtoScript II First Strand cDNA Synthesis Kit (New England Biolabs Inc). 2x master mixes were prepared using 2 μ L 10x Reverse Transcription buffers, 0.8 μ L 25x dinucleotide triphosphate (dNTP) mix (100 mM), 2 μ L 10x Reverse Transcription random primers, and 1 μ L MultiScribe Reverse Transcriptase. For a 20 μ L reaction mixture under the following conditions: 5.8 μ L master mix; 2 μ L of 2 μ g RNA sample; 12.2 μ L nuclease free water.

For analysis of gene expression, the real-time RT-PCR was performed in an Chai Real-Time PCR thermocycler with touchscreen (Chai Inc U.S.A) using Chai Green qPCR Master (Chai Inc U.S.A) following the manufacturer's instructions and procedure described by Livak and Schmittgen, (2001).



Table 1: Primer sequence

S/N	Gene	Forward (5–3')	Reverse (5–3')
1	MMP9	CCTCTGCATGAAGACGACATAA	GGTCAGGTTTAGAGCCACGA
2	GAPDH	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACGCCAGTA

3.5 Statistical analysis of data

All obtained raw data were analyzed using a one-way analysis of variance (ANOVA) by using the SPSS 25.0 computer program followed by post hoc Tukey HSD multiple comparisons to determine the statistically significant variations between the various parameters in the experimental replicates. A p-value of < 0.05 was considered statistically significant. Data were shown as means \pm SD. All graphs were generated using GraphPad Prism 8 (GraphPad Software Inc., San Diego, 189 CA, USA)

4 Results

All experimental groups showed no altered clinical observations or behavioral alterations except for Hg-exposed rats displayed a decreased activity, food consumption, and body weights with frequently increased urination when compared with the other experimental groups. Meanwhile, rats from the Hg-MOLE, Hg-FeNP and Hg-MOLE-FeNP groups were clinically normal and did not exhibit any signs of toxicity.

4.1 Characterization of green synthesized FeO-NPs

4.1.1 UV-visible spectroscopic analysis of FeO-NPs

The stimulation of the surface plasmon resonance of FeO-NPs gave the reaction solution its distinctive brown-red colour, which served as a useful spectroscopic hallmark of their production (Fig.1). Using a UV-Visible spectrophotometer, the reduction of ferric oxide was analysed spectroscopically. This revealed a 270 nm absorbance peak (Figure.2), which was unique to FeO-NPs.

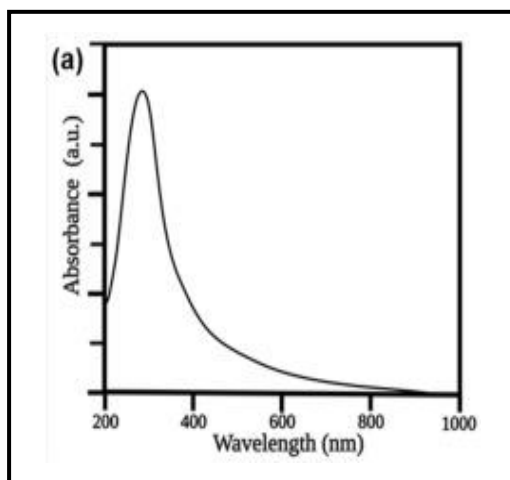


Figure 1: The UV-Visible absorption spectrum of green synthesised FeO-NPs.

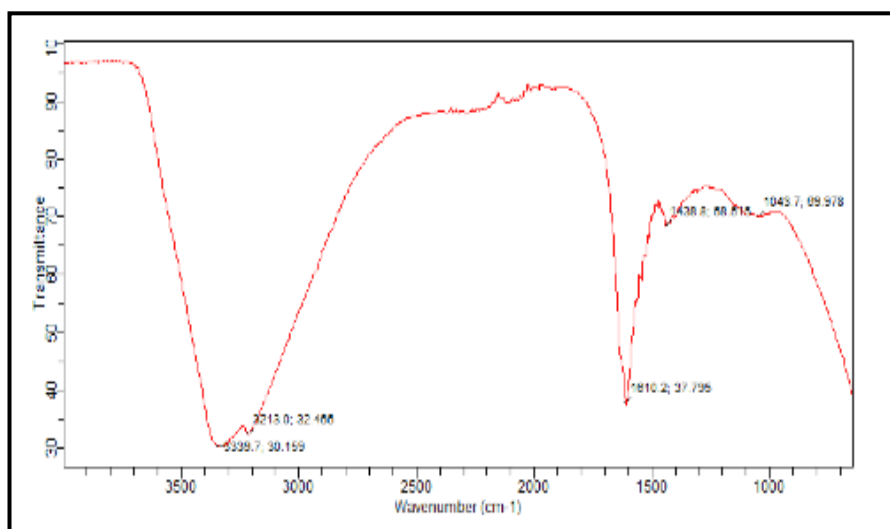


Figure 2: The FTIR spectra of FeO-NP

4.1.2 FTIR analysis of FeO-NPs

The FTIR spectra of the green synthesized FeO-NPs highlighted the functional groups present in FeO-NP (Figure 4.3). The peak that appeared near 660 cm^{-1} could be assigned to the stretching vibration of the Fe–O–Fe bond in FeO-NPs (Rahman et al., 2011). The peaks found in between 3400 and 3300 cm^{-1} , 1630 cm^{-1} , 1400 cm^{-1} and 1057 cm^{-1} were assigned to stretching vibration of O–H bond, stretching vibration C O bond, bending vibration of C–H and stretching vibration of C–N bond, respectively (Karade et al., 2019). The occurrence of these bonds can be attributed to the presence of the phenolic hydroxyl group, phenolic acids, terpenoids-phenols, and aliphatic amines.

4.1.3 XRD study of IONPs

The XRD measurement is frequently found to be a valuable analytical tool for determining the crystalline nature of freshly generated compounds and their phases. The XRD pattern of the FeO-NPs, clearly identified the fcc structure of α -Fe₂O₃ nanoparticles (JCPDS file, No. 89–2810) (51). During this measurement, a sequence of diffraction peaks was detected that were consistent with the theory. The prominent peaks of the XRD pattern suggest that the green synthesized FeO-NPs were well-crystallized α -Fe₂O₃ (Han et al., 2013).

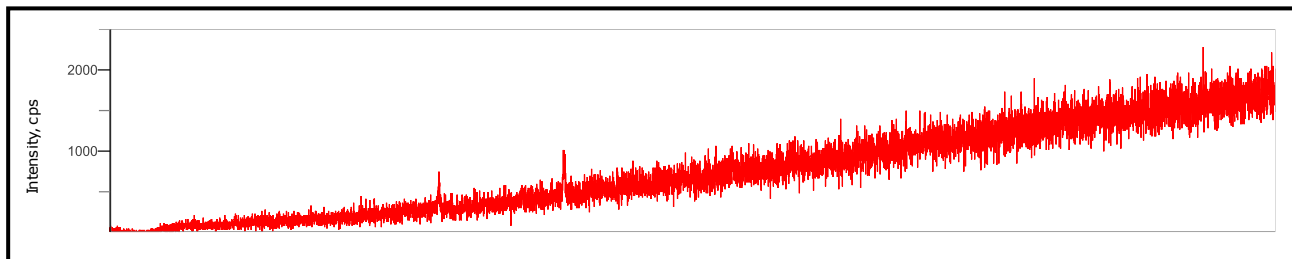


Figure 3: XRD pattern of green synthesized FeO-NPs

4.1.4 EDX observation of FeO-NPs

The EDX spectrum from one of the densely populated ferric oxide nanoparticles regions, obtained in the spot-profile mode, is shown in Figure 4.5 EDX spectra showed two distinct signals of iron (Fe) and oxygen (O) in synthesized FeO-NPs (Mukherjee et al., 2016). Peak related to any other element did not appear in the EDX spectrum, which confirmed that the synthesized FeO-NPs were made of Fe and O.

A Scanning Electron Microscopy (SEM) study was carried out to analyse the size and morphology of the synthesized FeO-NPs. The average size of these FeO-NPs was 5 ± 1 nm (Range: 3–10 nm) (Mukherjee et al., 2016). In nature, particles were found to be quasi-spherical and mono-disperse.

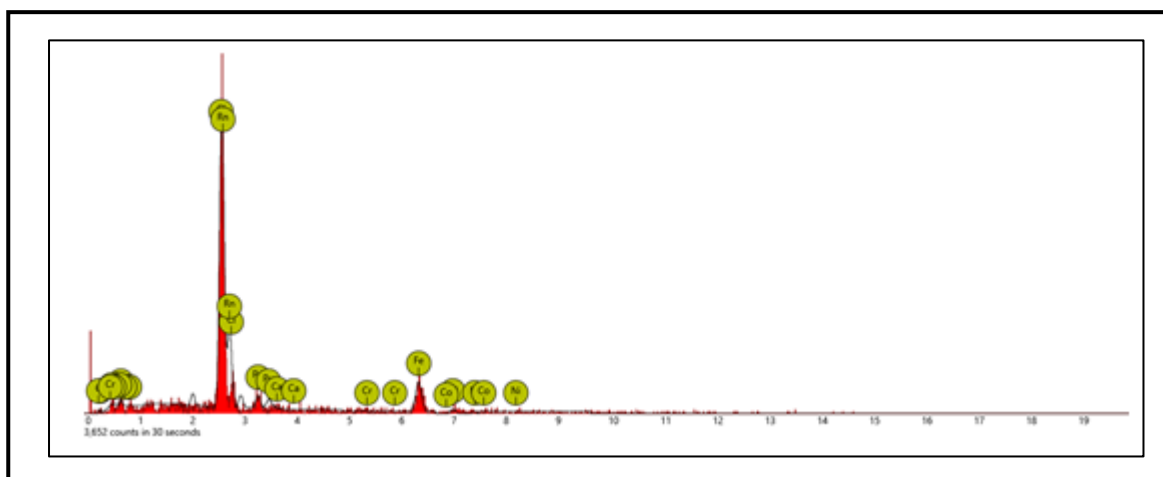


Figure 4: EDX spectrum of green synthesized FeO-NPs

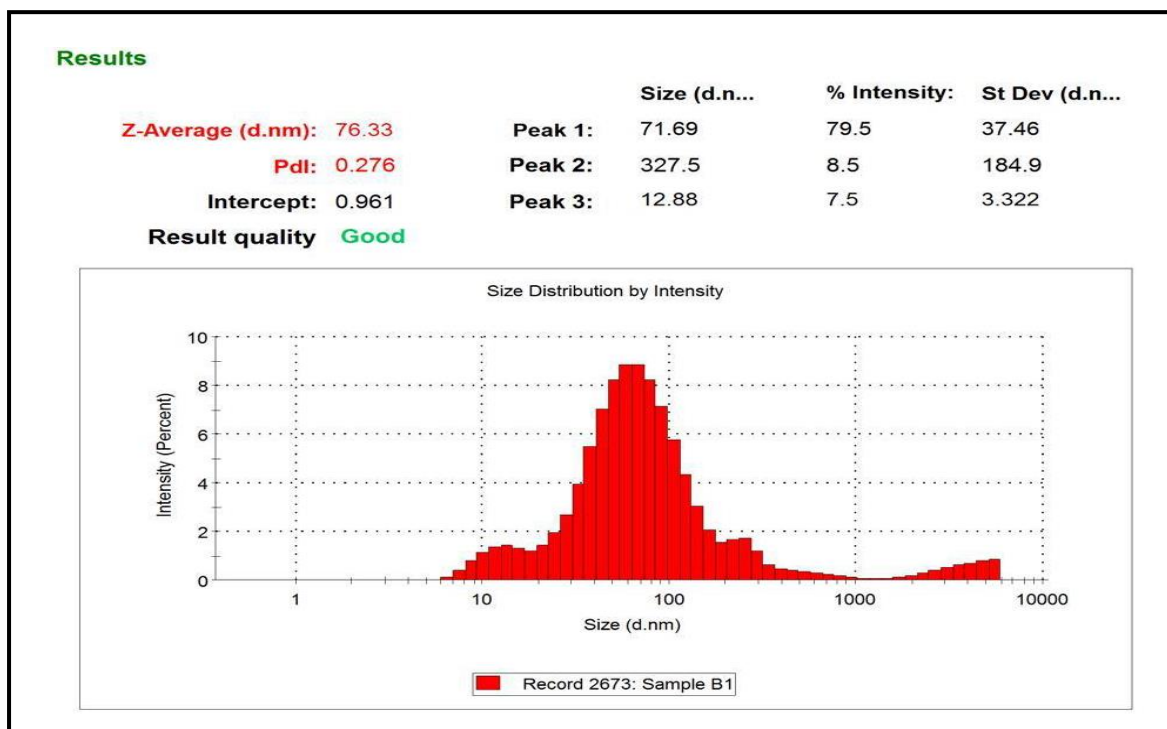


Figure 5: Particle size of green synthesized FeO-NPs

Figure 5 indicates the particle size distribution of the synthesized FeO-NPs using Zetasizer. The result shows the nanoparticles has Z-Average of 76.33 nm while the PDI is 0.276. The PDI value shows the synthesized iron nanoparticles is monodispersed which is much recommended for any particle to be used within the body system. However, nano particle sizes of ≤ 150 nm are considered tolerable for clinical application in systemic drug delivery (Danaei et al., 2018).

The morphology shows the FeO-NPs have rough surface and are composed of cavity-like structures similar to that which reported previously by (Devi et al. 2019).

4.1.5 Relative mRNA levels of apoptosis-related genes (Mmp9) in renal tissues

The probability of Hg-induced renal cell apoptosis was investigated via measuring the transcriptional levels of apoptosis-related gene (Mmp9) as shown in (Figure 5). Hg induced increase in apoptosis related gene in the renal tissues. Administration of MOLE, FeNP and MOLE-FeNP in the experimental rat shows reduced toxic effect. The effect was more pronounced in those groups that received MOLE-FeNP.

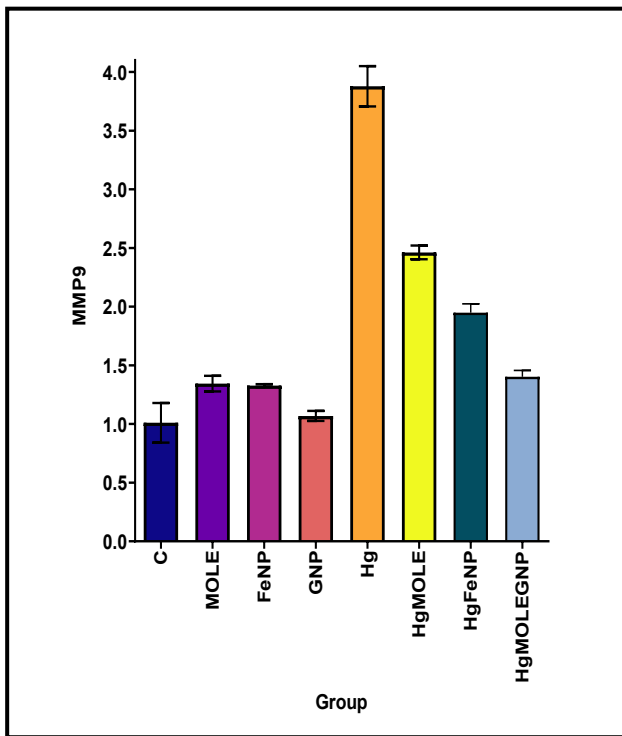


Figure 6: Transcriptional levels of (Mmp9) gene

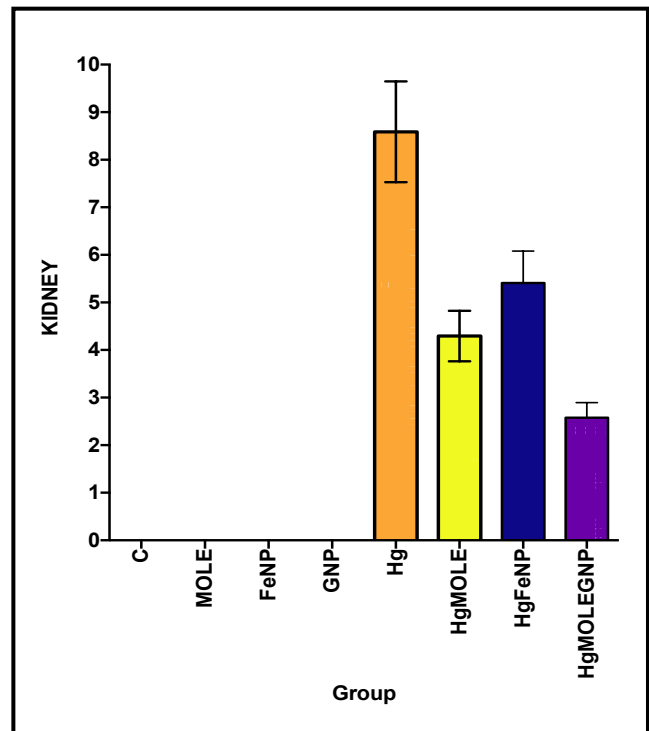


Figure 7: Expression of Mercury levels in the kidney

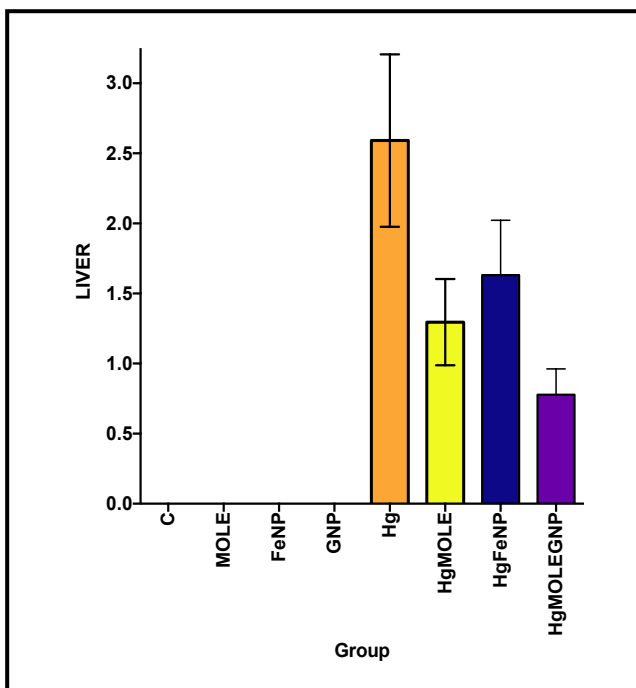


Figure 8: Expression of Mercury levels in the liver

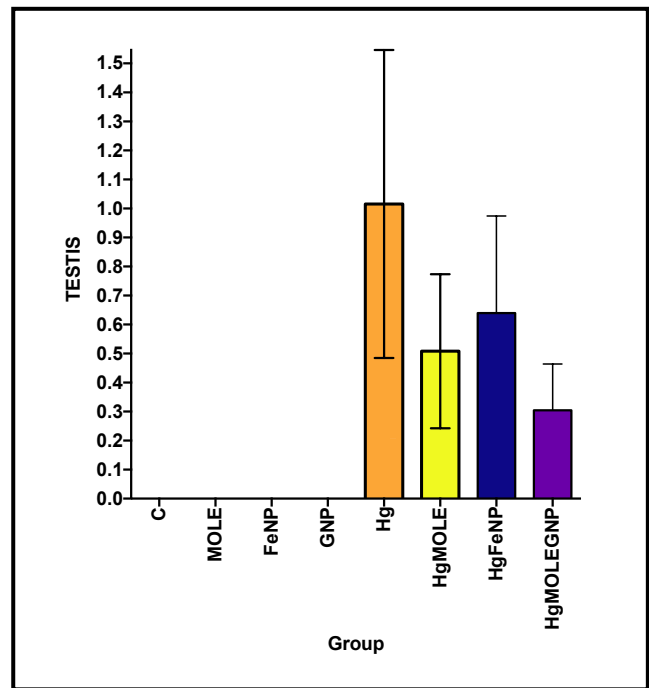


Figure 9: Expression of Mercury levels in the testis

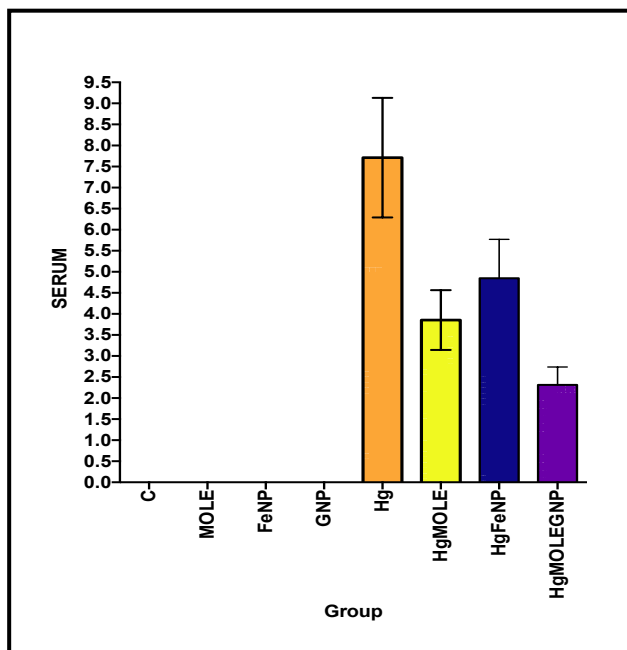


Figure 10: Expression of Mercury level in the serum

4.1.6 Serum and tissue mercury levels

The results of Figures 7, 8, 9 and 110, show that Hg was not detected in serum and tissues of kidneys, livers and serums of animals in control, MOLE, FeNP and MOLE-FeNP groups. However, there was significant increase in Hg levels in groups that received Hg +MOLE, Hg + FeNP and Hg + MOLE-FeNP. This implies the rats was free of Hg prior to the administration of Hg. Results of the above figure indicate that MOLE, FeNP and MOLE-FeNP (4.0, 5.5 and 2.5 against 8.0) all has the capacity of ameliorating Hg. Nevertheless, MOLE-FeNP (2.5 vs 8.0) has the most ability as shown above.

4.1.7 Immune response

Table 2, below present the immune responses of all the groups. The administration of Hg triggers a tremendous immune response in animal groups that received Hg, Hg-MOLE, Hg-FeNP and Hg-MOLE-FeNP respectively. There was significant increase in INF-gamma, IL-1B, TNF-alpha, TGF-beta1, NF-kB, IgM and IGF-1 in rats that were exposed to Hg indicating the presence of toxin. However, the effect was ameliorated with the administration of Hg-MOLE, Hg-FeNP and Hg-MOLE-FeNP. The greatest impact was felt in those that received MOLE-FeNP as they are close to the control group.



Table 2: Immune response

	Control	MOLE	FeNP	MOLE-FeNP	Hg	Hg-MOLE	Hg-FeNP	Hg-MOLE-FeNP
INF-y (ng/L)	30.826±	51.635±	48.963±	49.958±	102.331±	40.410±	38.319±	30.607±
	2.249	2.833	2.566	3.334	5.878	2.217	2.008	2.043
IL-1B (pg/ml)	6.080±	15.918±	16.167±	15.841±	34.306±	12.458±	12.653±	9.705±
	0.498	1.701	1.565	0.948	3.707	1.331	1.225	0.581
TNF-α (pg/ml)	18.423±	35.552±	33.140±	36.908±	75.541±	27.823±	25.935±	22.612±
	3.463	0.538	3.246	1.773	2.915	0.421	2.540	1.086
IL-6 (pg/ml)	163.382±	302.465±	307.550±	300.656±	613.706±	236.712±	240.691±	184.196±
	17.734	16.680	7.874	11.540	16.781	13.054	6.162	7.070
IL-10 (pg/ml)	2.261±	1.666±	1.389±	1.626±	0.544±	1.336±	1.482±	1.827±
	0.172	0.079	0.064	0.233	0.082	0.062	0.068	0.117
TGF-β1 (pg/ml)	9.244±	12.348±	12.517±	12.450±	27.204±	10.931±	11.221±	9.631±
	0.504	0.387	0.389	0.346	2.530	0.945	0.669	0.544
NF-κB (pg/ml)	0.057±	0.143±	0.179±	0.238±	1.420±	0.174±	0.172±	0.086±
	0.016	0.016	0.054	0.057	0.173	0.039	0.045	0.044
IgM	18.475±	24.678±	25.015±	24.880±	54.367±	21.846±	22.426±	18.887±
	1.007	0.772	0.777	0.691	5.056	1.888	1.337	0.527
IGF-1	6.955±	9.824±	9.958±	9.905±	21.643±	8.697±	8.928±	7.340±
	1.074	0.308	0.309	0.275	2.013	0.752	0.532	0.769

4.2 Discussion

From the results obtained above it was possible to synthesize green nanoparticles using *Moringa oleifera* leaf extract. The characterization of the green synthesized nanoparticles, revealed the presence of several function groups such as OH, C=O, C-N etc. these functional groups predicate the efficacy of the nanoparticles. This assertion was supported the work of Karade et al., (2019), who revealed that green synthesized FeO-NPs contain OH, C=O, C-N etc functional groups. And the assertion of Paz et al., (2014), that the presence of several functional group predicts the efficacy of the nanoparticles.

Moringa oleifera leaf extract is known for its health benefits, its effects were also felt in its ability to ameliorate toxic impact of mercury on the immune response in rats as shown in table 2 above. This again was supported by the work of Khalil et al., (2020) who revealed that *Moringa oleifera* leaf extract mediates against cell apoptosis and immune suppression.



The conjugate of MOLE and FeNPs which is the green synthesized iron nanoparticles proved more efficient in mediating against mercury induced Mmp9 gene expression as was indicated figure 5 above.

The probability of Hg-induced renal cell apoptosis was investigated via measuring the transcriptional levels of apoptosis-related gene (Mmp9) in the kidney. Hg increased the transcription of apoptosis related gene when administered as shown in figure 6. However, with the administration of MOLE, FeNP and MOLE-FeNP, the effect was ameliorated. The amelioration was most pronounced in the group that received MOLE-FeNP, showing that the green synthesized iron nanoparticles have the greatest potentials of suppressing Hg effect on Mmp9 gene. Figure 6, 7, 8 and 9 indicate that Hg was not detected in kidney liver, serum and testis in control, MOLE, FeNP and MOLE-FeNP groups prior to the administration of Hg.

The immune system of rats induced mercury was compromised when administrated with Hg resulting in increase in INR-gamma, IL-1B, TNF-alpha, IL-6, IL-10, TGF-beta1, NF-kB, IgM and IGF-1 activities in rats as shown in table 2 above. Nevertheless, with the introduction of MOLE, FeNP and MOLE-FeNP, the impact of mercury was reversed. The reversal was most prominent in those that received MOLE-FeNP as they are similar to the control group.

5 Conclusion and Recommendation

This study provides evidence that the green synthesized FeNPs using *Moringa oleifera* leaf extract protect against mercury induced immune responses and mmp-9 mRNA expression in rats. The results may be attributed to their potent antioxidant and anti-inflammatory properties. Thus, the result also revealed that green synthesized FeNPs have the potential of it being used as a remedy for mercury induced immune suppression and Mmp-9 mRNA expression.

We recommend that further researches be carried out on the histopathology and immunohistochemistry of the tissues mentioned above to ascertain the authenticity of our findings and if positive, we recommend that the green iron nanoparticles be commercialized as a supplement for mercury artisanal worker and others prone to mercury exposure.

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