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Research Article

Protective Effects of *Moringa oleifera* Leaf Extract and Quercetin Against Mercury-Induced Reproductive and Hepatic Toxicity in Rats

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ABSTRACT

Mercury chloride ($HgCl_2$) is a toxic heavy metal known to adversely affect various physiological systems, including the male reproductive system. This study evaluated the protective effects of *M. oleifera* extract and quercetin, against $HgCl_2$-induced reproductive and hepatic toxicity in experimental rats. Thirty (30) male Sprague Dawley rats were randomly assigned to six groups (n = 6): Control (no treatment), $HgCl_2$ (4 mg/kg), $HgCl_2$ + *M. oleifera* (30 mg/kg), $HgCl_2$ + quercetin (30 mg/kg), *M. oleifera* alone, and quercetin alone. All substances were administered orally for 30 days. Following treatment, blood and tissue samples (liver, testes and epididymis) were collected for biochemical, histological, and semen analyses. Rats exposed to $HgCl_2$ showed significant reductions in liver, testes, and epididymis weights, along with impaired sperm parameters (count, motility, viability) and decreased serum testosterone levels ($p < 0.05$). Co-treatment of $HgCl_2$ with *M. oleifera* or quercetin significantly improved these outcomes, restoring reproductive indices toward normal levels. Antioxidant activity was enhanced in the treatment groups, evidenced by significant increases in superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT), with a significant decrease in malondialdehyde (MDA), indicating reduced oxidative stress. Additionally, liver enzyme profiles and the TC/HDL cholesterol index improved significantly ($p < 0.05$). Histological evaluation revealed partial restoration of testicular architecture in treated groups, while epididymal tissues remained unaffected. These findings suggest that *M. oleifera* extract and quercetin mitigate mercury-induced testicular and oxidative damage, highlighting their potential as adjunct therapies in managing heavy metal-induced reproductive dysfunction.

Keywords: *Mercury chloride, M. oleifera extract, Quercetin, Reproductive parameters, Oxidative stress, Hepatotoxicity*

INTRODUCTION

Exposure to toxic substances- whether through environmental pollutants, pharmaceuticals, food, or food additives- poses a significant threat to public health (Miller *et al.,* 2024). Several environmental chemicals pose adverse health effects on humans particularly due to their pervasive nature and capacity to induce systemic toxicity (Aarab *et al.,* 2004; Tchounwou *et al.,* 2012; Mitra *et al.,* 2022). Mercury chloride ($HgCl_2$), a highly toxic inorganic form of mercury, has been extensively documented for its adverse effects on the male reproductive system, including impaired spermatogenesis, hormonal imbalances, and structural degeneration of testicular tissue (Martinez *et al.,* 2014; Briffa *et al.,* 2020; Lettieri *et al.,* 2022, Kushawaha *et al.,* 2025) These toxic effects are primarily mediated by the increase in the generation of reactive oxygen species (ROS), which lead to oxidative stress and lipid peroxidation (Boujbiha *et al.,* 2009). Many plant-derived dietary components with antioxidant properties are being studied as chemopreventive agents because of their capacity to neutralize free radicals (Jabłońska-Trypuć & Wiater, 2022). *Moringa oleifera*, often called the "miracle tree," is particularly rich in bioactive

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compounds such as flavonoids, polyphenols, and essential vitamins, all of which demonstrate antioxidant, anti-inflammatory, and cytoprotective effects (Debajyoti *et al.*, 2017; Tshabalala *et al.,* 2019). Aqueous extracts from the seeds, fruits, and leaves of *M. oleifera* contain bioactive constituents such as ellagic acid, quercetin, gallic acid, kaemferol, chlorogenic acid, ferulic acid, vanillin, and several vitamins (Padayachee & Baijnath, 2020; Pareek *et al.,* 2023). Quercetin, a widely distributed dietary flavonoid, is a key component in *M. oleifera* with strong antioxidant capacity, modulating several pathways involved in oxidative stress and inflammation (Vergara-Jimenez *et al.,* 2017; Carrillo-Martinez *et al.,* 2024).

Studies have shown that extracts of *M. oleifera* enhance male reproductive parameters like sperm concentration, motility, viability, and normal morphology in rats (Zade *et al.,* 2013; Dafaalla *et al.,* 2016). In addition, the ethanolic extract of *M. oleifera* leaves has been shown to improve sexual performance in stress-induced rats (Prabsattroo *et al.,* 2015). Similarly, quercetin has been reported to ameliorate sperm damage and improve sperm morphology in diabetic rats and in rats exposed to toxic compounds in crude oil vapour (Yelumalai *et al.,* 2019; Khazaeel *et al.,* 2022). While both *M. oleifera* and quercetin have been independently reported to confer protective effects against various toxicants (Shin *et al.,* 2015; Abarikwu, 2017a; Ahmed *et al.,* 2020; Jin *et al.,* 2024), there is a paucity of data on the comparative effects of *M. Oleifera* leaf extract and quercetin in ameliorating mercury-induced reproductive toxicity. Such comparisons could help elucidate whether the therapeutic benefits of the plant extract are predominantly due to quercetin alone or the result of synergistic interactions among its multiple constituents. This study, therefore, aims to evaluate and compare the protective effects of *M. oleifera* leaf extract and quercetin on mercury chloride-induced reproductive and hepatic toxicity in male rats.

MATERIALS AND METHODS

Chemical

Quercetin powder dietary supplement was acquired from Time Laboratories, Pocatella, United States**.** Mercury chloride a product of Sigma chemicals, was obtained from the Department of Biochemistry, University of Lagos, Lagos State, Nigeria and was dissolved in 10% of ethanol.

Experimental animals

Thirty adult Sprague Dawley male rats weighing between 140-160 g were used for this study. The animals were supplied by the Animal care facility of the College of Medicine University of Lagos, Lagos Nigeria. The animals were allowed to acclimatize for two weeks under normal room temperature before the study commenced. The animals were fed *ad libitum* with standard rat pelletized chow. The cages were maintained daily, and the rats were randomly assigned into six groups of six rats each after acclimatization. The University of Lagos Ethical Review Committee granted ethical approval with the approval number CMUL/ACUREC/08/23/1252, all experimental procedures were carried out in accordance with the Institutional Ethical Review Committee of the University. This study lasted for 30 days.

Animal groupings

Group I (Control): Received 10 mL/kg of distilled water orally.
Group II (Mercury chloride only): Received mercury chloride at a dose of 4 mg/kg body weight orally.
Group III (Mercury chloride + *Moringa* extract): Received mercury chloride (4 mg/kg) and *M. Oleifera* leaf extract (30 mg/kg) orally.
Group IV (Mercury chloride + Quercetin): Received mercury chloride (4 mg/kg) and quercetin (30 mg/kg) orally.
Group V (*Moringa* extract only): Received *M. Oleifera* leaf extract (30 mg/kg) orally.
Group VI (Quercetin only): Received quercetin (30 mg/kg) orally.

Collection of plant materials and plant extraction

The leaves of *M. oleifera* were procured from Mushin market at Mushin Area Council Lagos State, Nigeria and authenticated in the Department of Botany, University of Lagos with voucher number 9944 in the herbarium unit. The fresh leaves of *M. oleifera* were dried under room temperature for three (3) days and pulverized into powder form. The powdered form was soaked with 200 mls of distilled water and stored inside the fridge. While in the fridge it was turned/mixed for every six (6) hours for 72 hours. The mixture was then filtered, and the supernatant was oven dried under 40^oC and the extract was gotten.

Animal sacrifice

The animals were anaesthetized using an intraperitoneal injection of 25% (w/v) urethane and 1% (w/v) α-chloralose at a dose of 5 mL/kg body weight. Prior to anesthesia, feed was withdrawn for at least six hours to minimize complications during sample collection. Blood samples were collected via cardiac puncture into plain tubes, allowed to clot, and centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum was subsequently used for liver function tests, lipid profile analysis, and testosterone measurement. Organs including the liver, testes, and epididymis were excised, blotted, and weighed. The caudal epididymis was used for sperm analysis, while portions of the testes and epididymis were fixed in 10% formalin for histological evaluation. Additionally, the liver and testis tissues were homogenized in 0.1 M Phosphate Buffer, pH 7.4 included and used for antioxidant enzyme assays.

Sperm analysis and testosterone assay

Sperm analysis was conducted following the excision of the caudal epididymis, which was crushed in 1 mL of physiological saline to facilitate the release of spermatozoa. For sperm count, a 10 µL aliquot of the diluted sperm suspension was loaded into each chamber of a haemocytometer. After allowing the cells to settle for 5

minutes, spermatozoa were counted under a light microscope (Olympus CX21, Beijing, China) at 400× magnification. Sperm morphology was assessed according to the method described by Kruger *et al.*, (1987), with abnormalities classified as headless sperm, banana-shaped heads, bent necks, or bent tails, following the criteria outlined by Melissa, (2004). Sperm motility was estimated by adding 10 µL of the sperm suspension on a clean glass slide and examining the sample under a light microscope (400× magnification), as described by Zemjanis, (1970). Serum testosterone concentration was determined using ELISA kits from Elabscience, Wuhan, China), according to the manufacturer's instructions.

Estimation of malondialdehyde

The concentration of malondialdehyde (MDA) in the homogenized liver samples was determined using the thiobarbituric acid reactive substances (TBARS) assay, as described by Ohkawa *et al.,* (1979), with slight modifications.

Determination of antioxidant markers

The Sun and Zigman (1978) method was employed to determine the enzyme activity of superoxide dismutase (SOD) in the liver homogenates. Briefly, the assay was performed in 0.05 M sodium carbonate buffer (pH 10.3) and initiated by the addition of 0.3 mM epinephrine dissolved in 0.005 N HCl.

The activity of catalase (CAT) was measured following the method of Aebi (1984), which measures the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. The decrease in absorbance at 240 nm was monitored over time (1-2 minutes), and catalase activity was calculated using the molar extinction coefficient of H_2O_2.

Reduced glutathione (GSH) levels were estimated using the Ellman's reagent method, which is based on the formation of a yellow-colored complex between GSH and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The absorbance of the resulting chromophore was measured at 412 nm and quantified against a standard GSH curve (Gunzler, 1985). All absorbance readings were taken using a Process and General Instruments T70 UV/VIS spectrophotometer (Lutterworth, UK).

Lipid profile analysis

Serum lipid profile parameters were quantified using an automated chemistry analyzer (Mindray BS-120, Shenzhen, China) based on the spectrophotometric principle. Analyses were performed with commercially available diagnostic kits supplied by ERBA Diagnostics (Transasia Bio-Medicals Ltd., Mannheim, Germany).

Liver function test

Serum total bilirubin concentration was determined using a colorimetric method based on the diazo reaction. The assay employed the following reagents: R1 (sulphanilic acid), R2 (sodium nitrite), R3 (caffeine), and R4 (tartrate). In brief, 200 µL of R1 was added to a clean test tube, followed by 200 µL of serum, 50 µL of R2, and 1000 µL of R3. The mixture was thoroughly mixed and incubated at 25 °C for 10 minutes. Subsequently, 1000 µL of R4 was added, mixed, and further incubated at 25 °C. Absorbance was measured at 546 nm using a spectrophotometer. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using the colorimetric method described by Reitman and Frankel (1957). Absorbance was measured at 546 nm, and enzyme activities were calculated using a standard reference chart.

Histopathological analysis

Testes and epididymis samples were fixed in 10% buffered formaldehyde solution for a minimum of 24 hours prior to histopathological examination. Following fixation, tissues were dehydrated through a graded series of ethanol concentrations (70%, 80%, 90%, 95%, and 100%), cleared in xylene, and embedded in paraffin wax. Paraffin-embedded tissues were sectioned at a thickness of 5 µm using a rotary microtome and mounted on clean glass slides. The sections were then deparaffinized in xylene, rehydrated through descending grades of ethanol (100% to 70%), and rinsed in distilled water. Staining was performed using hematoxylin and eosin (H&E), and the sections were mounted with Canada balsam (Sigma, USA). Histological evaluation was carried out under a binocular light microscope, and representative photomicrographs were captured at ×100 magnification.

Statistical analysis

Data from this study were presented as mean ± standard error of the mean (SEM). The statistical analysis was performed using one-way analysis of variance (ANOVA) and multiple comparison was done using Bonferroni post hoc test. Differences were considered statistically significant at $p < 0.05$ using the GraphPad software (version 7).

RESULTS

Table 1 shows the effect of *M. oleifera* extract and quercetin supplementation on body and organ weights in mercury chloride-treated rats. There was no significant difference (p>0.05) in body weight across all groups. However, the weights of the liver and epididymis in the mercury chloride administered group were significantly decreased when compared with the control (p<0.05). $HgCl_2$ + Moringa supplemented rats had increased liver weight when compared with $HgCl_2$ group. No significant difference was observed in the testes weight across the groups.

Table 2 presents the sperm parameters and serum testosterone levels across the treatment groups. Mercury

chloride administration caused a significant reduction in sperm count compared to the control group ($p < 0.05$). However, co-treatment with *M. Oleifera* or quercetin significantly improved sperm count relative to the mercury chloride-only group ($p < 0.05$). Similar trends were observed for sperm motility, sperm viability, and serum testosterone levels. These parameters were significantly decreased in the mercury chloride, mercury chloride + moringa, and mercury chloride + quercetin groups compared to control ($p < 0.05$), but supplementation with moringa or quercetin significantly enhanced these values compared to mercury chloride alone ($p < 0.05$). Furthermore, the percentage of abnormal sperm cells increased significantly in all mercury-exposed groups compared to control. Notably, the co-administration of moringa or quercetin with mercury chloride resulted in a significant reduction in abnormal sperm morphology compared to the mercury chloride-only group.

Table 3 summarizes the testicular oxidative stress markers across the treatment groups. Mercury chloride exposure caused a significant increase in testicular malondialdehyde (MDA) levels compared to the control group ($p < 0.05$), indicating elevated lipid peroxidation. Co-treatment with *M. Oleifera* or quercetin significantly reduced MDA levels relative to the mercury chloride-only group ($p < 0.05$). Conversely, activities of superoxide dismutase (SOD), catalase (CAT), and levels of reduced glutathione (GSH)—were significantly decreased in the mercury chloride group compared to controls ($p < 0.05$). Notably, GSH levels were also significantly lower in the $HgCl_2$ + moringa group compared to control ($p < 0.05$). Supplementation with moringa or quercetin alongside mercury chloride significantly restored testicular SOD activity compared to mercury chloride alone ($p < 0.05$). Additionally, quercetin co-treatment significantly increased testicular GSH levels relative to the mercury chloride group ($p < 0.05$). Both moringa and quercetin-only groups exhibited higher GSH levels compared to all mercury chloride-exposed groups, including the co-treatment groups.

Table 4 presents the oxidative stress markers in the liver across treatment groups. Mercury chloride exposure significantly increased liver malondialdehyde (MDA) levels in the $HgCl_2$, $HgCl_2$ + *Moringa*, and $HgCl_2$ + quercetin groups compared to the control ($p < 0.05$). However, co-administration of moringa or quercetin with mercury chloride significantly reduced liver MDA levels relative to the $HgCl_2$-only group ($p < 0.05$). Activities of liver antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and levels of reduced glutathione (GSH) were significantly decreased in the mercury chloride group compared to control ($p < 0.05$). Notably, GSH levels were also significantly lower in the $HgCl_2$ + *Moringa* group compared to control ($p < 0.05$). Supplementation with *Moringa* and quercetin alongside mercury chloride significantly restored liver SOD and CAT activities compared to $HgCl_2$ alone ($p < 0.05$). Additionally, quercetin co-treatment significantly elevated liver GSH levels compared to the mercury chloride group, demonstrating superior antioxidant restoration relative to *Moringa*.

Table 1: Effect of *M. oleifera* Extract and Quercetin Supplementation on Body and Organ Weights in Mercury Chloride Treated Rats

	Control	$HgCl_2$	$HgCl_2$ + *Moringa*	$HgCl_2$ + Quercetin	*Moringa*	Quercetin
Initial weight (g)	148.4 ± 4.3	146.2 ± 1.8	150.8 ± 4.8	148.0 ± 3.5	149.6 ± 3.6	149.4 ± 1.2
Final weight (g)	174.6 ± 5.47	159.8 ± 9.74	163 ± 5.35	167.8 ± 9.29	169.6 ± 6.01	167.4 ± 5.29
Liver weight (g)	6.17 ± 0.18	4.82 ± 0.21*	6.00 ± 0.19#	5.51 ± 0.24	5.32 ± 0.21	5.61 ± 0.17
Testes weight (g)	2.73 ± 0.08	1.74 ± 0.28	2.65 ± 0.08	1.69 ± 0.09	2.18 ± 0.07	2.22 ± 0.05
Epididymis weight (g)	0.89 ± 0.04	0.55 ± 0.07*	0.88 ± 0.03#	0.75 ± 0.04#	1.07 ± 0.04#$!	0.98 ± 0.03#!

*Represent significant difference from control, # significant difference from $HgCl_2$, $ represent significant difference from $HgCl_2$ + Moringa and ! represent significant difference from $HgCl_2$ + Quercetin.

Table 2. Sperm Parameters and Serum Testosterone Levels of the Treatment Groups

	Control	$HgCl_2$	$HgCl_2$+ Moringa	$HgCl_2$+ Quercetin	Moringa	Quercetin
Sperm count (10^6)	103.8 ± 1.59	56.6 ± 1.11*	102.± 0.44#	96.0 ± 2.02#$	107.08 ± 1.55#!	114.1 ± 2.0*#$!^
Sperm Motility (%)	82.7 ± 0.89	41.3 ± 1.47*	71.6 ± 0.54*#	74.33 ± 0.89*#	90.66 ± 0.89*#$!	94.0 ± 0.71*#$!
Sperm Viability	78.0 ± 0.93	46.3 ± 1.47*	72.0 ± 0.35*#	69.0 ± 0.70*#	85.0 ± 1.27*#$!	97.6 ± 0.5*#$!^
Sperm Morphology (%)	0.67 ± 0.32	17.3 ± 0.54*	9.66 ± 0.54*#	6.0 ± 0.35*#	3.0 ± 0.35*#$!	2.0 ± 0.35#$!
Testosterone levels (ng/mL)	1.22 ± 0.05	0.23 ± 0.01*	0.77 ± 0.02*#	0.82 ± 0.04*#	1.15 ± 0.03#$!	1.29 ± 0.03#$!^

*Represent significant difference from control, # significant difference from $HgCl_2$, $ represent significant difference from $HgCl_2$ + Moringa, ! represent significant difference from $HgCl_2$ + Quercetin and ^ significant difference from Moringa.

Table 3. Testicular Oxidative Stress Indices of the Treatment Groups

	Control	$HgCl_2$	$HgCl_2$ + *Moringa*	$HgCl_2$ + Quercetin	*Moringa*	Quercetin
MDA (µmol/ml)	2.45 ± 0.10	4.02 ± 0.10*	2.86 ± 0.14#	2.79 ± 0.14#	2.64 ± 0.08#	2.42 ± 0.04#
SOD (µmol/ml/min/mg Pro)	2.43 ± 0.01	1.88 ± 0.02*	2.30 ± 0.04#	2.29 ± 0.04#	2.34 ± 0.03#	2.34 ± 0.08#
CAT (µmol/ml/min/mg Pro)	13.20 ± 0.1	10.56 ± 0.5*	11.73 ± 0.7	11.76 ± 0.2	13.13 ± 0.3#	13.01 ± 0.2#
GSH (µmol/ml)	65.15 ± 1.2	56.15 ± 0.8*	57.93 ± 0.6*	59.68± 0.4*#	62.91 ± 0.4#$	65.33 ± 0.8#$!

* Represent significant difference from control, # significant difference from $HgCl_2$, $ represent significant difference from $HgCl_2$ + Moringa and, ! represent significant difference from $HgCl_2$ + Quercetin. MDA= malondialdehyde, SOD= superoxide dismutase, CAT= catalase and GSH= Reduced glutathione

Table 4. Liver Oxidative Stress Indices of the Treatment Groups

	Control	$HgCl_2$	$HgCl_2$ + *Moringa*	$HgCl_2$ + Quercetin	*Moringa*	Quercetin
MDA (μmol/ml)	2.59 ± 0.05	5.53 ± 0.13*	4.35± 0.24*#	4.15± 0.2*#	2.5± 0.18#$!	2.68 ± 0.18#$!
SOD (μmol/ml/min/mg Pro)	2.94 ± 0.06	1.61 ± 0.06*	2.96 ± 0.15#	2.68 ± 0.03#	3.32± 0.12#!	3.17 ± 0.11#!
CAT (μmol/ml/min/mg Pro)	14.73± 0.81	8.71 ± 0.96*	15.71± 1.65#	12.20± 0.9#	16.6± 0.55#!	18.56 ± 0.87#!
GSH (μmol/ml)	48.44 ± 1.6	33.67 ± 1.7*	37.70 ± 1.1*	40.1± 0.7*#	53.5± 1.2#$!	46.88± 1.8#$!^

*Represent significant difference from control, # significant difference from $HgCl_2$, $ represent significant difference from $HgCl_2$ + Moringa, ! represent significant difference from $HgCl_2$ + Quercetin and ^ significant difference from Moringa. MDA= malondialdehyde, SOD= superoxide dismutase, CAT= catalase and GSH= Reduced glutathione

Table 5 illustrates the effects of moringa and quercetin supplementation on lipid profile parameters in mercury chloride-treated rats. There was no significant difference in total serum cholesterol across all groups compared to the control. However, the mercury chloride group showed a significant decrease in HDL levels and an increase in LDL levels and the total cholesterol to HDL (TC/HDL) ratio compared to the control ($p < 0.05$). Supplementation with moringa and quercetin in the $HgCl_2$-treated groups significantly lowered LDL levels and the TC/HDL ratio compared to the $HgCl_2$-only group ($p < 0.05$), indicating improved lipid profile regulation.

Table 6 presents the effects of moringa and quercetin supplementation on liver enzymes and bilirubin levels in mercury chloride-treated rats. The $HgCl_2$ group exhibited significantly elevated levels of AST, ALT, and bilirubin compared to the control ($p < 0.05$). Supplementation with both moringa and quercetin in the $HgCl_2$-treated groups significantly reduced AST, ALT, and bilirubin levels relative to the $HgCl_2$-only group; however, ALT and bilirubin levels remained significantly higher than control values ($p < 0.05$). Administration of moringa or quercetin alone lowered AST, ALT, and bilirubin levels compared to the $HgCl_2$-treated groups, with quercetin alone demonstrating a more pronounced effect than *Moringa*.

Table 5. Lipid Profile Parameters of the Treatment Groups

	Control	$HgCl_2$	$HgCl_2$ + *Moringa*	$HgCl_2$ + Quercetin	*Moringa*	Quercetin
TC (mmol/L)	1.68 ± 0.10	1.95 ± 0.14	1.60 ± 0.09	1.80 ± 0.09	1.70 ± 0.03	1.70 ± 0.10
HDL (mmol/L)	0.80 ± 0.03	0.53 ± 0.05*	0.59 ± 0.04	0.66 ± 0.07	0.80 ± 0.07#	0.71 ± 0.05
LDL (mmol/L)	0.75 ± 0.02	1.16 ± 0.14*	0.81 ± 0.02#	0.68 ± 0.07#	0.65 ± 0.08#	0.70 ± 0.06#
Triglycerides (mmol/L)	1.01 ± 0.05	1.16 ± 0.07	1.10 ± 0.06	1.0 ± 0.10	0.83 ± 0.03	0.90 ± 0.09#
TC/HDL	2.17 ± 0.12	3.94 ± 0.26*	2.81 ± 0.32#	2.78 ± 0.15#	2.23 ± 0.24#	2.42 ± 0.16#

*Represent significant difference from control, # significant difference from $HgCl_2$ TC=Total cholesterol, LDL= Low density Lipoprotein, HDL= High density lipoprotein

Table 6. Liver enzymes and Bilirubin in the treatment groups

	Control	$HgCl_2$	$HgCl_2$ + *Moringa*	$HgCl_2$ + Quercetin	*Moringa*	Quercetin
AST (μ/L)	200.8 ± 5.50	244.7± 11.4*	202.7 ± 4.85#	210.9 ± 3.24#	179.5 ± 1.3#	174.3 ± 2.10*#
ALT (μ/L)	61.63 ± 1.11	74.5 ± 1.22*	69.6 ± 0.89*#	68.28 ± 1.2*#	65.13 ± 0.7#	64.9 ± 0.85#$
Bilirubin (μmol/L)	2.05 ± 0.23	5.30 ± 0.07*	3.85 ± 0.16*#	3.78 ± 0.2*#	2.67± 0.2#$!	2.01 ± 0.16#$!

*Represent significant difference from control, # significant difference from $HgCl_2$, $ represent significant difference from $HgCl_2$ + Moringa and, ! represent significant difference from $HgCl_2$ + Quercetin.

Figure 1 shows the photomicrograph of the testicular tissue in rats administered moringa, quercetin and $HgCl_2$. The control group shows normal testicular architecture with seminiferous tubules lined by complete spermatogenic series numerous luminal spermatozoa are present and no histological abnormalities observed. In the $HgCl_2$ group, seminiferous tubules lined by spermatogenic cells with numerous distorted luminal spermatozoa and vascular congestion were seen. The tubules of the $HgCl_2$ + Moringa group show distorted luminal spermatozoa and congested blood vessels admixed with floccular pink material, indicative of mild interstitial edema. In the $HgCl_2$ + Quercetin group there was a similar observation to what was seen in the $HgCl_2$ + Moringa group, with vascular congestion and interstitial edema observed. The sections for the Moringa and Quercetin only groups showed properties similar to those of the control.

Figure 2 shows the photomicrograph of the epididymal tissue in rats administered moringa, quercetin and $HgCl_2$. The control group showed normal epididymal architecture. The lumen is surrounded by pseudostratified columnar epithelium with stereocilia and smooth muscle layers. Numerous luminal spermatozoa are present. No abnormalities observed. The $HgCl_2$ group showed normal histological appearance. Epithelial integrity and smooth muscle structure preserved, with abundant luminal spermatozoa. No pathological changes noted. The $HgCl_2$ + Moringa group had preserved histoarchitecture with no observable abnormalities. Epithelium, stereocilia, and luminal spermatozoa appear normal. The $HgCl_2$ + Quercetin group showed no histological alterations. Normal pseudostratified epithelium, intact smooth muscle, and adequate luminal spermatozoa. The moringa only group showed epididymal tissue that generally appears normal, though some tubules show mild vascular congestion. Spermatozoa are present within the lumen. Overall findings are within normal limits. Lastly the quercetin only group showed normal histological presentation with well-organized

epithelium, intact smooth muscle, and abundant spermatozoa. No abnormalities observed.

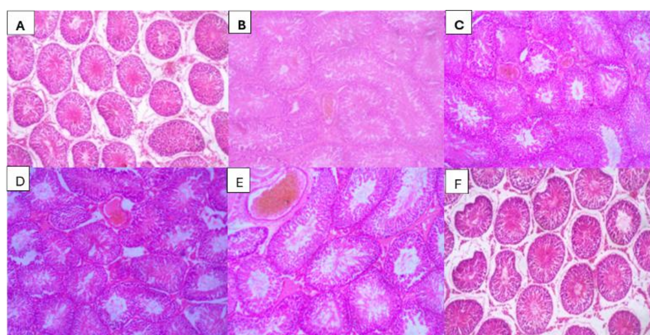

Figure 1. Photomicrograph of the Testicular Tissue of Rats Treated with *Moringa* and Quercetin in Mercury Chloride Induced Toxicity.
H and E Stain X 100. A= control, B= $HgCl_2$, Group, C= $HgCl_2$. + Moringa, D= $HgCl_2$. + Quercetin, E= Moringa only and F= Quercetin only

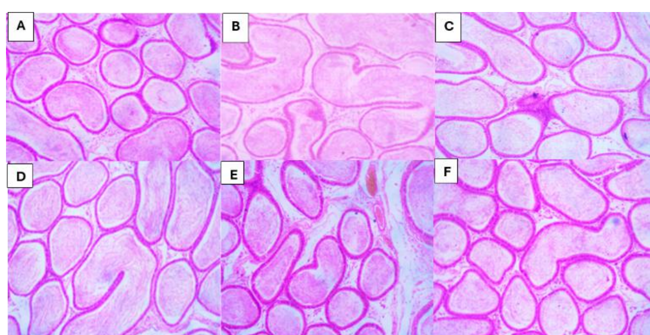

Figure 2: Photomicrograph of the Epididymal Tissue of Rats Treated with Moringa and Quercetin in Mercury Chloride Induced Toxicity.
H and E Stain X 100. A= control, B= $HgCl_2$, Group, C= $HgCl_2$. + Moringa, D= $HgCl_2$. + Quercetin, E= Moringa only and F= Quercetin only

DISCUSSION

The present study investigated the protective effects of *M. Oleifera* and quercetin against mercury chloride ($HgCl_2$)-induced male reproductive toxicity in rats. Studies has shown that mercury chloride, a well-known environmental toxicant, is associated impaired spermatogenesis, hormonal imbalances, and structural degeneration of testicular tissue. Consistent with previous studies, findings from this study showed $HgCl_2$ resulted in impaired sperm parameters as evidenced by reduced sperm count, decreased motility, and an increased incidence of morphological abnormalities (Martinez *et al.,* 2014; Briffa *et al.,* 2020; Lettieri *et al.,* 2022). These findings align with previous studies demonstrating that $HgCl_2$ induces oxidative stress, disrupts spermatogenesis by causing shrinkage of the seminiferous tubules and Leydig cells. Thus, reducing sperm count, sperm motility, and testosterone concentration. (El-Desoky *et al.,* 2013, Kandemir *et al.,* 2020; Shalan, 2022). The observed decrease in sperm motility and increase in abnormal forms, such as bent tails and headless sperm, are indicative of compromised testicular function and epididymal maturation (Shalan 2022, Akarsu *et al.,* 2024). Interestingly, treatment with *M. Oleifera* and quercetin markedly improved sperm parameters, reflecting their antioxidant, anti-inflammatory and protective properties. Studies using rutin, syringic acid, N-Acetylcysteine, gallic acid and vitamin E, with strong antioxidant properties have conferred protection on the testes and liver of rats induced with mercury chloride (Shalan, 2022; Vanithasri and Jagadeesan, 2023, Akarsu *et al.,* 2024). Co-administration of these agents restored sperm count and motility toward control values and reduced the frequency of morphological defects, suggesting a reversal of $HgCl_2$-induced reproductive toxicity. Notably, animals treated with *M. oleifera* or quercetin alone did not show any adverse alterations in sperm quality, further supporting their potential as fertility-enhancing and cytoprotective compounds.

The findings of this study showed that mercury chloride significantly elevated oxidative stress in testicular and liver tissues, as evidenced by increased MDA levels, a marker of lipid peroxidation. Simultaneously, there was a decrease in antioxidant enzymes SOD, CAT, and GSH. These essential findings are consistent with Akarsu et al. (2024), who reported that $HgCl_2$ exposure elevated MDA levels while reducing SOD, CAT, GPx activities, and GSH levels in rat testicular tissue. Similarly, Kandemir *et al.,* (2020), reported that $HgCl_2$ treatment increased malondialdehyde (MDA) levels, tumour necrosis factor-α (TNF-α) and cyclooxygenase-2 (COX-2) expressions, thus leading to necrosis and degeneration of spermatogonia. The male reproductive organs which are crucial for continuity of generations, are among the first tissues to be damaged by toxic substances (Massanyi *et al.,* 2020) Mercury can cross biological membranes and readily forms organo-mercury complexes with proteins, leading to the generation of reactive oxygen species and triggering oxidative stress in organisms exposed to $HgCl_2$. Therefore, antioxidant compounds may provide protective effects against $HgCl_2$-induced toxicity (Abarikwu, *et al.,* 2017b). In this study, co-treatment with *M. Oleifera* and quercetin effectively attenuated these changes, suggesting potent antioxidative effects. Both agents significantly lowered MDA levels and restored antioxidant enzyme activity toward normal values, however, the effects of quercetin were more pronounced and could be linked to their rich phytochemical contents (Debajyoti *et al.,* 2017; Oyewopo *et al.,* 2021; Carrillo-Martinez *et al.,* 2024). The observed biochemical recovery aligns with histological and sperm parameter improvements, reinforcing the role of oxidative stress in $HgCl_2$-induced reproductive and hepatic toxicity, and the therapeutic potential of these two natural antioxidants.

Dyslipidemia and increased liver enzymes were reported in the $HgCl_2$ group. Elevated LDL, along with a reduction in HDL, resulted in an increased TC/HDL index. Elevated levels of liver enzymes and bilirubin are associated with impaired liver function. This confirms previous reports that mercury chloride induces hepatotoxicity and compromises liver function in rats (Goudarzi *et al.,* 2017; Caglayan *et al.,* 2019). Previous studies have shown that $HgCl_2$ is associated with dyslipidemia and lipid profile disturbances, a risk factor for cardiovascular diseases (Kang *et al.,* 2021; Abasilim *et al.,* 2023). Remarkably, co-treatment with *M. Oleifera* and

quercetin reduced these effects, as evidenced by significant reductions in LDL levels, and TC/HDL index. These findings are consistent with the lipid-lowering and hepatoprotective properties previously reported for both *M. oleifera* and quercetin (Vergara-Jimenez *et al.,* 2017, Asgari-Kafrani *et al.,* 2020). Previous toxicology studies have shown that $HgCl_2$ induced increased liver enzymes, which were associated with cellular necrosis and increased membrane permeability (Verril *et al.,* 1997; Li *et al.,* 2021). Consistently, both natural antioxidants, *M. oleifera* and quercetin reduced these elevated enzyme levels, suggesting hepatoprotective properties. This could be due to their bioactive components, which enhance scavenging of ROS, stabilizing the cell membranes, and enhancement of endogenous antioxidant defenses, thus preventing hepatocyte damage.

Histological examination revealed that $HgCl_2$ altered testicular structure, showing distorted seminiferous tubules and vascular congestion accompanied by interstitial oedema. These findings are consistent with mercury's toxic effects on reproductive tissues, likely mediated through oxidative stress and inflammation, which impair spermatogenesis and compromise testicular microcirculation (Orisakwe *et al.,* 2001, Briffa *et al.,* 2020; Lettieri *et al.,* 2022). In contrast, epididymal histology remained largely unaffected, with preserved pseudostratified columnar epithelium, and normal luminal sperm content across all groups, suggesting a relative resistance of epididymal tissue to acute mercury toxicity under the experimental conditions. Testicular damage was mitigated by both *M. oleifera* and quercetin evidenced by improved tubular histoarchitecture and reduction in vascular congestion and oedema. These histological improvements correlated with enhanced sperm parameters and reduced biochemical markers of oxidative stress, reinforcing the protective role of these natural compounds in maintaining male reproductive tissue integrity against mercury-induced toxicity. In addition, animals treated with quercetin or *M. oleifera* alone showed histological features comparable to the control group, suggesting that both agents may exert inherent protective effects on reproductive tissues, even in the absence of toxic insult, with the actions of quercetin slightly pronounced than those of *M. oleifera*.

CONCLUSION

This study established that *M. oleifera* crude extract and quercetin, an active compound found in *M. oleifera* ameliorated the $HgCl_2$ reproductive and hepatic toxicity by improving sperm count, motility and morphology disrupted by mercury chloride. In addition, these natural antioxidants reduced liver enzyme levels and improved lipid profiles. The study also noted that the effects of the pure active compound were slightly better than those of the crude extract of *M. oleifera*. This suggests that both agents may exert inherent protective effects on reproductive and hepatic tissues in the presence or absence of toxic insult.

AUTHORS' CONTRIBUTIONS

GOO and BOI conceptualized the research. Method and formal analysis were carried out by GOO and JOS, Investigation by GOO and JOS, Data curation by GOO, BOI, Original draft preparation GOO and JOS, Writing, reviewing and editing by GOO and BOI, Supervision by GOO and BOI. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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