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Reproductive toxicity induced by polyethylene glycol and possible protective mechanism of *Moringa oleifera* in male rats

Hussein Kamil Awad[1], Emam Atiyah Ibadi[2], and Ashraf Ayyal Mutar Alrashedi[1]

[1]Unit of Environment and Prevention of Pollution, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq.

[2]Department of Environment, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq.

Hussein Kamil Awad (Unit of Environment and Prevention of Pollution, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq. Orcid No: 0009-0002-7601-1446 Email: hussein.Kamel@qu.edu.iq
Emam Atiyah Ibadi (Department of Environment, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq. Orcid No: 0000-0003-4758-6814 Email: emam.atiyah@qu.edu.iq
Ashraf Ayyal Mutar Alrashedi (Unit of Environment and Prevention of Pollution, College of Science, University of Al-Qadisiyah, Diwaniyah, Iraq. Email: Ashraf.ayyal@qu.edu.iq

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Abstract

Polyethylene glycol (PEG) has been used in many pharmaceuticals as a food additive and drug delivery mechanism as a catalyst. *Moringa oleifera* is considered one of the World's most valuable trees, as almost every part of the Moringa tree can be used for food, and medication and is rich in phytochemicals. The present study aims to determine the reproductive toxicity of polyethylene glycol and the protective role of *Moringa oleifera* in male rats. Animals were divided into six groups as follows: control group, MOLE (200 mg/ kg) group, PEG (50 mg/kg) group, PEG (50 mg/kg) + MOLE, PEG (100 mg/kg) group, and PEG (100 mg/kg) group + MOLE. Rats were orally administered their respective doses daily for 45 days. The results showed that treatment with both doses of PEG caused a significant increase in DNA fragmentation, TNFα, IL-6, TBARS, GPx, GST, SOD, Catalase, dead sperm, and total abnormal compared to the control group. While, both doses of PEG caused a significant decrease in mtTFA, PGC-1α, P53, GR, GSH, motile, and viability sperm compared to the control group. The results clearly show that the effect of PEG is dose-dependent. The presence of MOLE with PEG in the combination groups showed a positive protective effect against reproductive toxicity induced by PEG. From these results, MOLE could be used as a protective agent against the reproductive damage caused by PEG.

Keywords: Polyethylene glycol; *Moringa oleifera*; Reproductive toxicity; gene expression of peroxisome proliferator-activated

Abbreviations: TNFα, Tumor necrosis factor-alpha; IL-6, interleukin 6.

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Introduction

In food, medication, and cosmetics, polyethylene glycols (PEG) are polyether molecules that are frequently employed as additives, PEG are polymers made from ethylene oxide, composed of a frequent unit of – (O – CH 2 – CH2) –, or its hydrophilic oligomers **(1).**

Millions of tons of polyethylene glycol are manufactured worldwide and most of them will reach conventional sewage disposal systems after industrial utilization **(2).** According to the metabolic mechanism of PEG comprised sequential oxidation via alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) **(3).**

Moringa oleifera Lam (syn. M. pterygosperma Gaertn.) commonly known as moringa, drumstick, and horseradish, is a small, fast-growing ornamental tree that originates from India **(4).** Different parts of this plant contain a profile of important minerals and are a good source of protein, vitamins, β-carotene, amino acids, and various phenolics. The Moringa plant provides a rich and rare combination of zeatin, quercetin, β- sitosterol, caffeoylquinic acid and kaempferol. possess antitumor, anti-inflammatory, cholesterol-lowering, antioxidant, antidiabetic, and hepatoprotective **(5).**

Moringa leaves are an impending source of native antioxidants, leaf excerpts accomplished of sifting peroxyl and peroxyl extremists **(6).**

2. Materials and Methods

2.1. Tested compounds

The supplier was Central Drug House Ltd. in New Delhi, India, and the product was polyethylene glycol 1500, purity "99.9%".

The National Research Center - Egypt - Cairo - Dokki supplied the *moringa oleifera leaf* extract (MOLE).

2.2. Experimental groups and animals

2.3. Test subjects & animals

The present investigation utilized wister male rats. The Faculty of Medicine at Alexandria University in Alexandria, Egypt provided the animals. The Institutional Animal Care and Use Committee (ALEXU-IACUC) approved this study and determined it complied with all applicable regulations. Animals were kept in stainless steel wire cages with adequate nutrition (food and drink available whenever needed) and an airtight environment (25-5 °C, 50–70% relative humidity). 14 days for adaptation. Six equally sized groups of rats (n = 6) were created. Group 1 is the control group, which consists of untreated, healthy rats. Group 2 consists of rats given MOLE 200 mg/kg. Group 3, PEG 50 mg/kg. Group 4, PEG 50 mg/kg+ MOLE. Group 5 is PEG 100 mg/kg and Group 6 is PEG 100 mg/kg + MOLE. For 45 days in a row, doses were administered daily. The above dosages of Moringa oleifera leaf extract and polyethylene glycol were calculated on **(7)** and **(8),** one-to-one.

2.4. Collect blood samples and get tissues ready

Isoflurane was utilized to anesthetize the rats before they were sacrificed after 45 days had passed. Blood samples were drawn into Heparin-based anticoagulant test tubes. Centrifuging at 860× g for 20 minutes later, plasma was extracted from the blood kept must be analyzed at -80 °C. Remove the testes right away, wash them with a fluid sodium chloride, and remove them with caution. adherent fat and interconnected tissues. Unconnectedly, a portion of the testes were in ice-cold sucrose buffer (0.25 M) and homogenized (10%, w/v). To remove the cell debris, the sample was centrifuged at 10,000 g for 20 min at 4 °C, and the supernatant was removed and kept at -80 °C for analysis. remaining parameters.

2.5. Organ and body weights

Rat's primary & ultimate organ We recorded the weights. Additionally, after the testes were removed, their weight was immediately recorded and dried on tissue paper.

2.6. PGC-1 and mtTFA quantitative expression measurement in testes

Tissue was performed using quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR). First, the total RNA was isolated from the tissues, then the isolated RNA was reverse-transcribed. According to the manufacturer's recommendations, quantitative expression analysis of PGC-1**(9)** and mtTFA **(10)** in testes tissue was carried out. PGC-1; F- 5'-AAACTTGCTAGCGGTCCTCA-3; R- 5'-TGGCTGGTGCCAGTAAGAG-3; mtTFA; F- 5'-CCTTCGATTTTCCACAGAACA-3; and GAPDH; F- 5'-GGGTGTGAACCACGAGAAATA-3; and R- 5'-AGTTGTCATGGATGACCTTGG3; are the primary sequences that were utilised.

2.7. Inspect of DNA breakages

DNA breaks, such as marker cellular death, were measured using the approach of **(11).**

2.8. ELISA stands for enzyme-linked immunosorbent assay.

p53 (cat. no. ELR-p53-1), TNF- (cat. no. ab100785), and IL-6 (cat. no. ab100772 Ray Biotech, Inc.) concentrations were measured in the homogenates of reproductive organs. by the use of corresponding ELISA kits (Abcam) following the manufacturer's instructions.

2.9. Markers of oxidative stress

Tetramethoxypropane (TMP) serial concentrations were used to produce a standard curve, which was then used to calculate the level of TBARS **(12)**. Nitric oxide end products (N.O) nitrite and nitrate, which are present in the deproteinized samples as nitrite and nitrate, were quantified using the Griess reaction. The addition of nitrate reductase, an NADPH-dependent enzyme, to the Griess reaction, produced nitrite instead of nitrate. The assay process had two actions: the first needed sulphanilic acid to be diazotized with nitrite ions., and the subsequent required attaching this resultant pink metabolite with a diamine, producing a detectable amount of it at 540 nm. inclination of the standard curve, which was created using successive concentrations of nitrite sodium, was used near calculate the level of N.O Superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST), and catalase (CAT) activities in tissue homogenates were measured using colorimetric kits (Bio diagnostic, Egypt) following the manufacturer's instructions and using particular standards for each parameter. After protein precipitation, reduced glutathione concentration was measured making use of a reagent for metaphosphoric acid. The assay relied on the oxidation of GSH by 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), which generated GSSG and 5-thio-2-nitrobenzoic acid (TNB). The amount of GSH in the sample had a direct relationship to TNB conversion rate production, which remained measured at 412 nm. By keeping track of the absorbance change, the rate of TNB production was observed. It was determined that this was 412 nm per minute (A/min). A GSH standard curve was used to determine the total glutathione content in the samples, as well as the outcomes then represented as nmol/g tissue by splitting the glutathione a sample's concentration divided by its weight (in grams) the tissue used to create the instance **(13).**

2.10. Semen characteristics

To assess sperm count, sperm motility, and sperm morphology, an epididymis was produced. According to the technique of **(14)**, Using an Olympus microscope and the Computer Assisted Semen Analysis (CASA System; Germany) were employed. Each rat's 200 spermatozoa were individually evaluated and classified as normal or abnormal using the stringent sperm morphological standards outlined in **(15)**. According to **(16)** methodology, acrosome integrity was evaluated.

2.11. Determination of reproductive hormone Testosterone

Using an ELISA kit for the quantitative determination of testosterone in rat plasma from DRG International Co. in the U.S.A., testosterone levels were determined. The assays were done strictly according to **(17)**.

2.12 Statistical evaluation

All of the parameters' mean and standard errors were calculated, and the findings were reported as mean standard error. A one-way analysis of variance (ANOVA) and Duncan multiple comparisons were used to analyze the data. **(18)** found a statistically significant difference at P 0.05.

Percentage of change = $(mean\ of\ treatment - mean\ of\ control)/(mean\ of\ control) * 100$

Results and Discussion

Information was gathered that showed MOLE and PEG therapy had negligible effects on reproductive organ weights **(Figure 1).**

The results revealed that, as compared to the control group, PEG 50 and PEG 100 significantly reduced mtTFA and PGC1-α levels in the testes. The data also revealed a substantial increase in DNA fragmentation in each of the PEG50 & PEG100 treated sets when contrasted with the control group. In the same regard, MOLE treatment of rats greatly elevated mtTFA and PGC-1α when compared to PEG (50 and 100mg/kg) and MOLE. In contrast, MOLE treatment considerably reduced DNA fragmentation in the presence of PEG 50 + MOLE and PEG 100 + MOLE when compared to PEG 50 and PEG 100 **(Table 1 & Figure 2).**

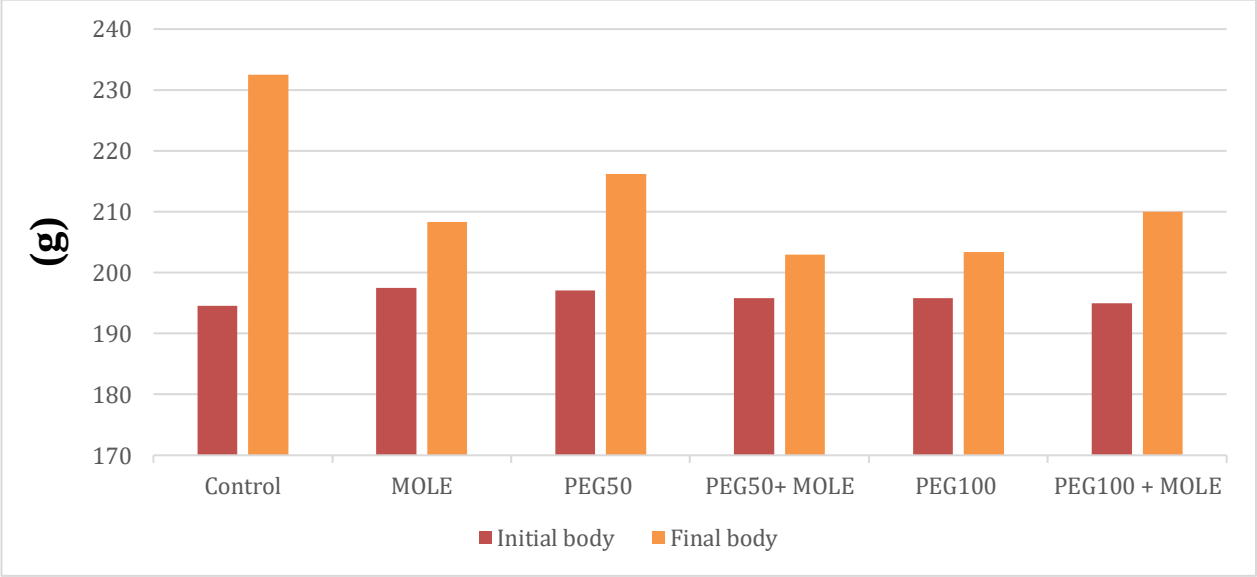

Figure (1): Effects on Male Rats Initial and Final Body Weight of Polyethylene Glycols and *Moringa Oleifera* Leaf Extract

Table (1): Effects of PEG and *MOLE* on (mtTFA), (PGC-1α) &DNA fragmentation in testes of rats, male

group experiments	Parameters		
	mtTFA (Fold Change)	PGC1-α (Fold Change)	DNA fragmentation
Control	1.00±0.15^{ab}	1.00±0.13^{a}	13.47±0.53^{c}
MOLE	1.20±0.05^{ab} (20%)	1.09±0.12^{a} (9%)	12.79±0.87^{c} (-87.21%)
PEG (50 mg/kg)	0.59±0.06^{c} (-41%)	0.42±0.07^{c} (-58%)	24.31±1.37^{ab} (-75.06%)
PEG (50 mg/kg) + MOLE	1.01±0.05^{a} (1 %)	0.88±0.07^{b} (-12%)	15.88±0.96^{c} (-84.12%)
PEG (100 mg/kg)	0.45±0.04^{c} (-55%)	0.35±0.03^{c} (-65%)	27.33±1.830^{a} (-72.67%)
PEG (100 mg/kg) + MOLE	0.69±0.03^{c} (-31%)	0.57±0.03^{bc} (-43%)	22.09±0.67^{b} (-77.91%)

The results are expressed as (Mean ± SE)

^{abc}Mean values contained a column Significant difference existed between superscript letters that weren't shared., p< 0.05 MOLE; dose of *Moringa oleifera* leaves extract (200 mg/kg), PEG 50; dose of Polyethylene glycols (50 mg/kg), PEG 100; dose of Polyethylene glycols (100 mg/kg).

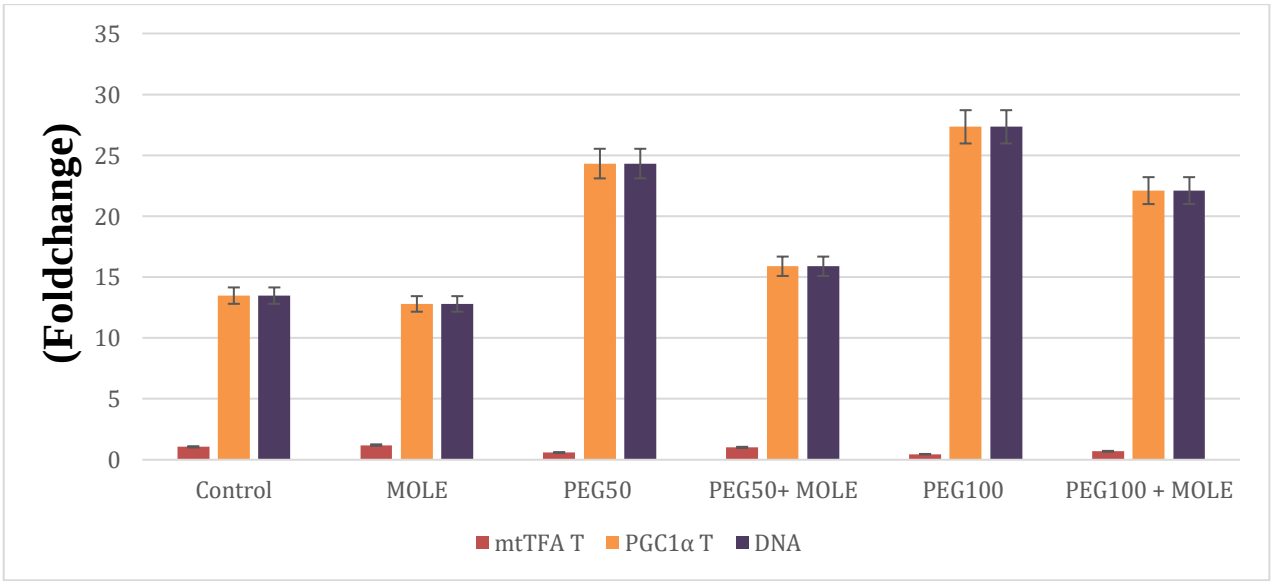

Figure (2) Effects of PEG and MOLE on (mtTFA), (PGC-1α) and DNA fragmentation in testes of male rats

The regulation of mitochondrial biogenesis, adaptive thermogenesis, antioxidant defenses, and cellular respiration are just a few of the crucial biological processes that PGC1-α has been shown to control, PGC1-α does this by activating downstream target genes like mtTFA and antioxidant enzyme genes **(19)**. In the current work, the dose-dependent downregulation of mtTFA and PGC1-α expression in testicular tissues of PEG-treated rats may be a sign of diminished mtDNA replication and transcription, as well as mitochondrial biogenesis, which may result in mitochondrial malfunction.

To bolster this hypothesis, it has been shown that exposure to environmental toxins impairs mitochondrial function, mostly as a result of alterations in the mitochondrial membrane's permeability **(20)**.

Pro-inflammatory cytokines (TNF- α & IL-6), in testes, were found, according to results provided in **(Table 2 and Figure 3)** Comparing the PEG 50 & PEG 100 groups to the temoin group, the outcomes dramatically improved. Rats getting MOLE treatment were significantly fewer when compared to PEG 50 + MOLE & PEG 100 + MOLE as well as PEG 50 & PEG 100 alone. However relative to the control group, P53 in the testes considerably decreased in both PEG 50 & PEG 100. Additionally, P53 altered insignificantly in PEG 100 + MOLE compared to PEG 100 but P53 increased significantly in PEG 50 + MOLE when compared to PEG 50 **(21)**. showed that (TNF-α & IL-6) increased with treatment PEG & decreased with MOLE and, at the same time it decreased P53 with a group of PEG and increased through treatment MOLE, this is in line with our results.

Table (2): Effects of PEG and *MOLE*on expression of tumor necrosis factors α (TNFα), expression of interleukin 6 (IL-6), and expression of tumor suppressor P53 (P53) in testes of male rats

group experiments	Parameters		
	TNF-α (ng/gm)	IL-6 (ng/gm)	P53 (ng/gm)
Control	5.01±0.39^{d}	1.9±0.13^{e}	6.26±0.50^{b}
MOLE	3.91±0.27^{d} (291%)	2.14±0.01^{e} (114%)	6.16±0.51^{b} (516%)
PEG (50 mg/kg)	15.82±0.69^{b} (1.48%)	9. 71±0.54^{c} (871%)	4.63±0.25^{c} (363%)
PEG (50 mg/kg) + MOLE	8.58±0.31^{c} (758%)	5.006±0.27^{d} (400.6%)	6.12±0.28^{b} (512%)
PEG (100 mg/kg)	27.37±0.75^{a} (2.63%)	19.78±0.97^{a} (1.87%)	3.80±0.02^{c} (280%)
PEG (100 mg/kg) + MOLE	14.70±0.82^{b} (1.37%)	12.10±0.42^{b} (1.11%)	4.44±0.25^{c} (344%)

The results are expressed as (Mean ± SE)

^{abc}Mean values contained a column Superscript letters that weren't shared were noticeably different., p< 0.05 MOLE; dose of *Moringa oleifera* leaves extract (200 mg/kg), PEG 50; dose of Polyethylene glycols (50 mg/kg), PEG 100; dose of Polyethylene glycols (100 mg/kg).

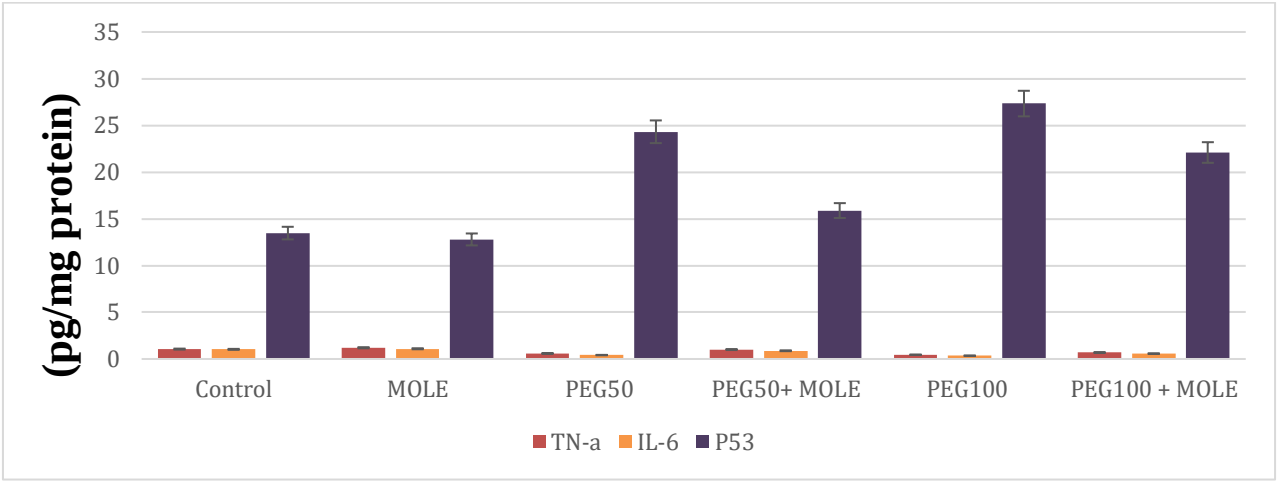

Figure (3): Effects of PEG and *MOLE* (TNFα), 6 (IL-6) and (P53) in testes of male rats

The observed results in N.O. insignificant changes in PEG 50 and significantly increased PEG100 treated groups when compared to the control group. During this time, rats were given MOLE extract insignificantly changed in PEG 50 + MOLE & PEG 100 + MOLE when equated to PEG 50 and PEG 100, As opposed to that, results indicate a meaningfully higher level of TBARS in the PEG50 & PEG100 handled groups as compared to the control group. contrasted with PEG 50 & PEG 100, one-to-one, the TBARS in rats treated with MOLE dramatically decreased. In contrast, the PEG50 and PEG100 treated groups showed no appreciable differences and a considerable rise in comparison to the control group. Comparing PEG 50 MOLE & PEG 100 MOLE to PEG 50 & PEG 100, the treatment of rats with MOLE extract did not significantly alter either **(Table 3 and Figure 4).**

Following the current finding, (22) observed that mice fed with Moringa oleifera leaf extract experienced a considerable reduction in TBARS.

The acquired results demonstrated a considerable reduction in (GR) in the PEG50 & PEG100 as contrasted with the control group, and the treated groups **(Table 4 & Figure 5).** In comparison to PEG 50 & PEG 100, MOLE treatment of rats significantly raised (GR) in PEG 50 + MOLE and PEG 100 + MOLE. Comparatively to the control group, the levels of (GPx), (GST), (SOD), & Catalase considerably increased in the PEG50 and PEG100 treated groups. When compared to PEG 50 and PEG 100, the (GPx), (GST), and (PEG 100 + MOLE, respectively, in the rats treated with MOLE dramatically decreased. Comparatively to the control group, (GSH) considerably decreased in the PEG50 and PEG100 treatment groups. Rats treated with MOLE experienced no appreciable change in GSH in PEG 50 + MOLE compared to PEG 50, but there was a considerable rise in GSH in PEG 100 + MOLE equated to PEG 100**.**

Table (3): effect of PEG and MOLE on nitric oxide and thiobarbituric acid reactive substances testes male rats

Experimental Groups	Nitric oxide (mU/mg protein)	TBARS (µmol/g protein)
Control	78.03±1.27[b]	1.03±0.03[d]
MOLE	73.63±1.55[b] (-5.63%)	1.07±0.032[d] (3.88%)
PEG (50 mg/kg)	77.88±1.78[b] (-0.19%)	1.52±0.04[c] (47.57%)
PEG (50 mg/kg) + MOLE	78.54±1.66[b] (0.65%)	1.13±0.05[d] (9.70%)
PEG (100 mg/kg)	90.32±4.18[a] (15.75%)	2.54±0.09[a] (146.6%)
PEG (100 mg/kg) + MOLE	74.10±2.86[b] (-5.03%)	1.76±0.06[b] (70.87%)

The results are expressed as (Mean ± SE)

[abc]Mean internal values a column Superscript letters that weren't shared were noticeably different., p< 0.05 MOLE; dose of *Moringa oleifera* leaves extract (200 mg/kg), PEG 50; dose of Polyethylene glycols (50 mg/kg), PEG 100; dose of Polyethylene glycols (100 mg/kg)

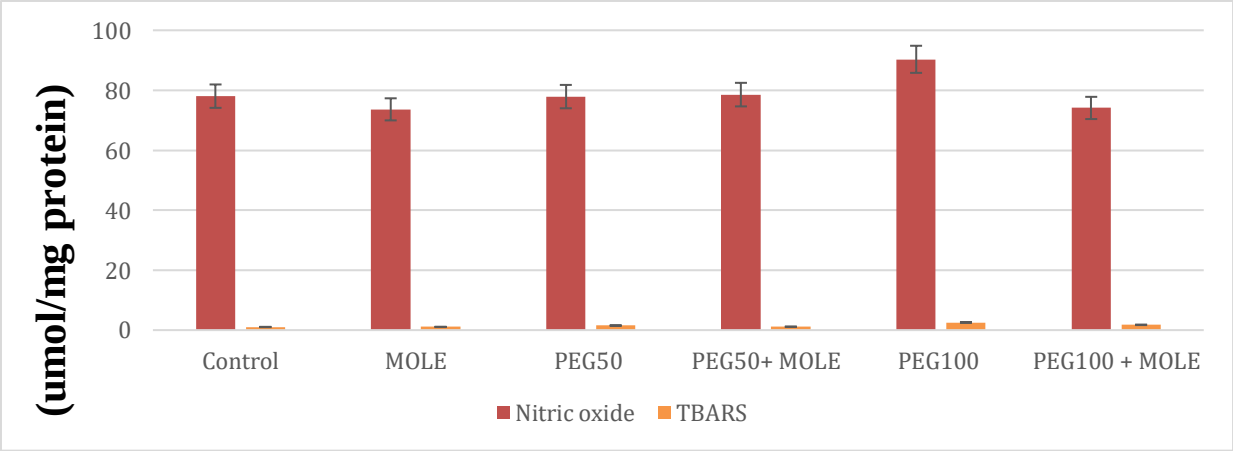

Figure (4): effect of PEG and MOLE on nitric oxide and thiobarbituric acid reactive substances testes male rats

Table 4: (GR), (GPx), (GST), (GSH), (SOD), and catalase responses to (PEG) and (MOLE)in testes

Experimental groups	Parameters					
	GR (IU/g protein)	GPx (IU/g protein)	GST (IU/g protein)	GSH (µmole/g m protein)	SOD (IU/g protein)	Catalase (IU/g protein)
Control	53.35±2.73^{a}	67.40±1.81^{d}	7.06±0.15^{c}	7.01±0.20^{a}	1.25 ±6.37^{c}	2.39±42.75^{d}
MOLE	40.19±1.58^{c} (-24.66%)	34.00±1.08^{e} (-49.55%)	5.76±0.19^{d} (-18.41%)	6.02±0.23^{b} (-14.12%)	1.26±14.26^{c} (0.8%)	2.40±61.73^{d} (0.41%)
PEG50	29.16±1.46^{d} (-45.34%)	97.68±1.005^{b} (44.92%)	10.31±0.20^{b} (46.03%)	4.74±0.17^{c} (-32.38%)	1.33±14.66^{b} (6.4%)	2.98±30.1^{c} (24.68%)
PEG50+MOLE	45.38±1.59^{b} (-14.77%)	66.35±1.41^{d} (-1.55%)	7.004±0.25^{c} (-0.79%)	4.25±0.14^{c} (-39.37%)	1.27±30.20^{c} (1.6%)	2.49±53.40^{d} (4.18%)
PEG100	25.47±0.64^{d} (-52.25%)	125.2±4.30^{a} (85.75%)	14.58±0.20^{a} (106.51%)	2.91±0.09^{e} (-58.48%)	1.43±9.93^{a} (14.4%)	3.38±18.61^{a} (41.42%)
PEG100+MOLE	36.47±1.13^{c} (-31.64%)	79.55±2.001^{c} (18.02%)	7.49±0.43^{c} (6.09%)	3.62±0.13^{d} (-48.35%)	1.35±24.1^{b} (8%)	3.15±34.56^{b} (31.79%)

The results are expressed as (Mean ± SE)

^{abc}Mean values contained a column Significant differences existed when common superscript letters were absent., p< 0.05 MOLE; dose of *Moringa oleifera* leaves extract (200 mg/kg), PEG 50; dose of Polyethylene glycols (50 mg/kg), PEG 100; dose of Polyethylene glycols (100 mg/kg)

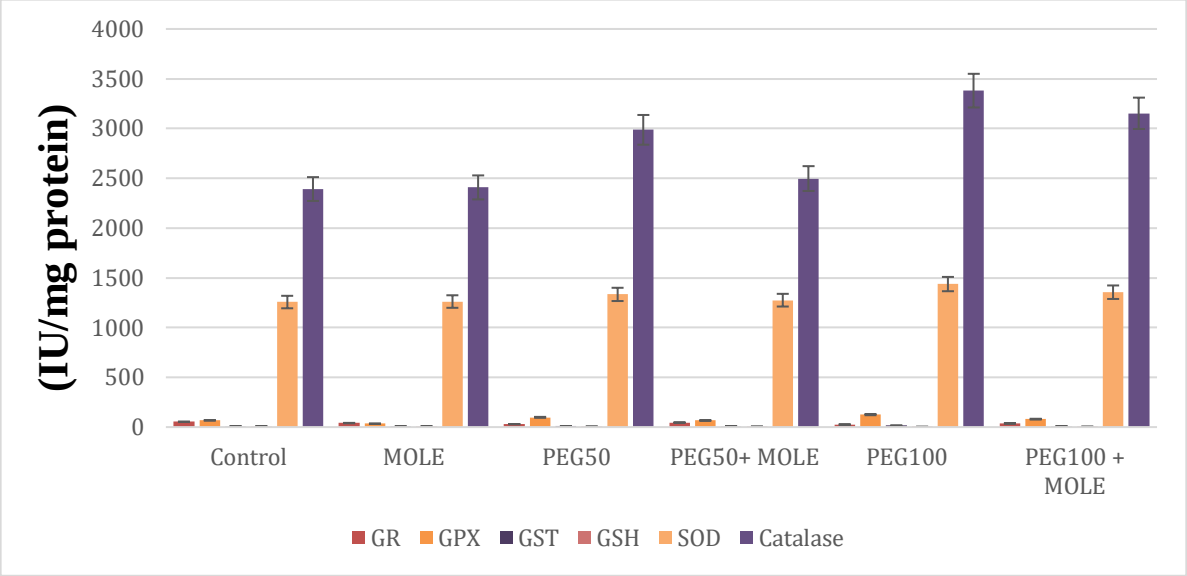

Figure 5: (GR), (GPx), (GST), (GSH), (SOD), and catalase responses to (PEG) and (MOLE)in testes

Aqueous extract of Moringa seeds (AEMS) results revealed a significant decrease in catalase and SOD, which is consistent with the most recent findings, according to **(23)**. indicated that Moringa oleifera leaves extract (MOLEE) Results showed catalase, glutathione peroxidase (GPx) insignificantly changed and SOD, GST, and GSH significantly increased when compared with the controls, results are non-line with the current results **(24)**. However, treatment with Moringa oleifera improved sperm parameters, testes morphology, antioxidant markers, and hormone assessments.

The outcomes displayed in comparison to the control group in **(Table 5 & Figure 6)** demonstrate a significant reduction in motile sperms in both PEG 50 and PEG 100. The number of motile sperms, however, increased dramatically in PEG 50+ MOLE when compared to PEG 50 & decreased slightly in PEG 100 + MOLE when compared to PEG 100. Additionally, the results indicate that, contrasted with the control group, sperm viability meaningfully decreased in both the PEG 50 & PEG 100 groups. Sperm viability, however, only little changed in PEG 100 + MOLE when compared to PEG 100 & dramatically improved in PEG 50+ MOLE & PEG 50 when compared to PEG 50. Comparatively, to the control group, dead sperm dramatically raised in both PEG 50 & PEG 100. As a result, dead sperm dramatically decreased in PEG 50+ MOLE as compared to PEG 50, while it barely altered in PEG 100+ MOLE. Additionally, both PEG 50 and PEG 100 considerably increased the number of abnormal sperm heads in comparison to the control group. Meanwhile, aberrant sperm head insignificantly altered in PEG 50+ MOLE and compared with PEG 50, and significantly decreased in PEG100 +MOLE when compared with PEG 100. When compared to the control group, abnormal sperm neck underwent no alteration in PEG 50 and a considerable increase in PEG 100. However, as compared to PEG 50 + MOLE and PEG 50, the aberrant sperm neck showed no significant change, while PEG 100 + MOLE and PEG 100 showed a considerable decrease. Comparing PEG 50 and PEG 100 to the control group, abnormal sperm tail considerably increased in both. In contrast, when PEG 50 + MOLE and PEG 100 + MOLE were compared to PEG 50 and PEG 100, the aberrant sperm tail considerably decreased in each of these treatments. Results are presented in and demonstrate no significant changes in altered acrosome in PEG 50 and a considerable rise in PEG 100 when compared to the control group. In contrast,

altered acrosome did not change considerably in PEG 50 + MOLE when compared to PEG 50 & significantly decreased in PEG 100 + MOLE when equated to PEG 100.

Oxidative phosphorylation occurs in sperm mitochondria and supplies energy for sperm movement **(25)**. Early stages of mammalian spermatogenesis require normal mitochondrial function **(26)**. During spermatogenesis, abnormal sperm mitochondrial function was linked to infertility. Because nuclear transcription factors co-regulate it, the mitochondrial genome functions in a somewhat autonomous manner. According to **(27)**, mtTFA is expressed during spermatogenesis up till the early and late spermatocyte stages.

The obtained results indicate that testosterone levels in the PEG50 and PEG100 treated compared to the control group were significantly greater. As compared to PEG 50 and PEG 100, testosterone levels in rats treated with MOLE considerably decreased in both PEG 50 MOLE and PEG 100 MOLE **(Table 6 and Figure 7).**

Following the most recent research, **(28)** stated that the event substantially improved the mean value testosterone serum levels.

Table (5): Effects of PEG and *MOLE* on Sperm Characteristics in Male Rats

Experimental groups	Parameters					
	Motile sperms (%)	Sperm viability (%)	Dead sperms (%)	Total abnormal sperm (%)	DNA abnormal Sperm (%)	altered acrosome (%)
Control	78.8±0.8^{a}	73.8±0.8a	21.2±0.8b	4.8±0.2^{cd}	4.8±0.37^{d}	5.0±0.316^{c}
MOLE	73.4±1.7ab (7.24%)	68.6±1.66ab (6.76%)	26.6±1.7ab (2.56%)	4.3±0.2^{d} (318.3%)	4.2±0.2^{d} (318.3%)	4.8±0.37^{c} (380%)
PEG (50 mg/kg)	66.8±1.24^{b} (6.58%)	61.6±1.36^{b} (6.76%)	33.2±1.24^{a} (3.22%)	6.0±0.31bc (500%)	6.6±0.51^{c} (500%)	5.8±0.58^{bc} (480%)
PEG (50 mg/kg) +MOLE	72.8±1.98^{ab} (7.18%)	67.6±2.18^{ab} (6.66%)	27.2±1.98^{ab} (2.62%)	5.8±0.37^{bc} (480%)	5.6±0.81^{cd} (480%)	6.4±0.6^{bc} (540%)
PEG (100 mg /kg)	68.6±2.80^{b} (6.76%)	62.8±2.88^{b} (6.18%)	31.4±2.80^{a} (3.04%)	8.8±0.96^{a} (780%)	12.8±0.73^{a} (780%)	9.8±1.39^{a} (880%)
PEG (100 mg /kg) +MOLE	69±3.28^{b} (6.8%)	63.2±3.45^{b} (6.22%)	31±3.28^{a} (3.00%)	7.2±0.37^{ab} (620%)	9.0±0.31^{b} (620%)	7.8±0.37^{ab} (680%)

The results are expressed as (Mean ± SE)

^{abc}Mean internal values a column Superscript letters that weren't shared were noticeably different., p< 0.05 MOLE; dose of *Moringa oleifera* leaves extract (200 mg/kg), PEG 50; dose of Polyethylene glycols (50 mg/kg), PEG 100; dose of Polyethylene glycols (100 mg/kg)

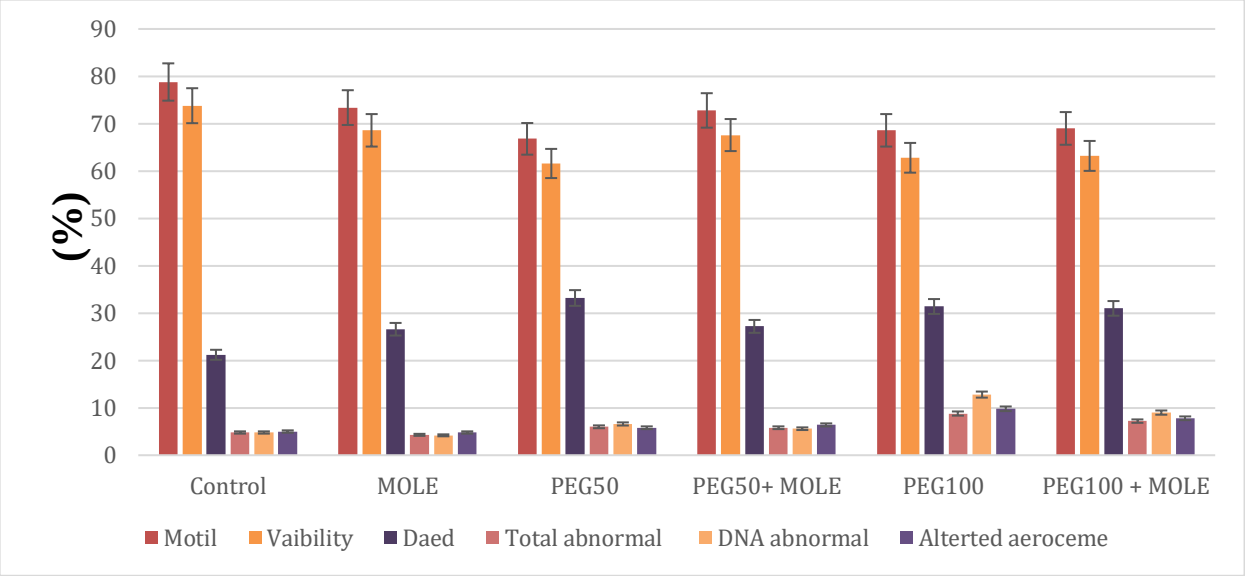

Figure (6): Effects of PEG and MOLE on Sperm Characteristics in Male Rats

Table (6): Effects of PEG and *MOLE* on testosterone among male rats' blood plasma

group experiments	Testosterone (ng/mL)
Control	0.41±0.03[b]
MOLE	0.42±0.01[b] (-99.56%)
PEG (50 mg/kg)	0.58±0.06[a] (-99.42%)
PEG (50 mg/kg) +MOLE	0.46±0.04[b] (-99.54%)
PEG (100 mg/kg)	0.69±0.02[b] (-99,59%)
PEG (100 mg/kg) +MOLE	0.59±0.004[c] (-99.90%)

The results are expressed as (Mean ± SE)

[abc]Mean values contained a column Superscript letters that weren't shared were noticeably different., p< 0.05 MOLE; dose of *Moringa oleifera* leaves extract (200 mg/kg), PEG 50; dose of Polyethylene glycols (50 mg/kg), PEG 100; dose of Polyethylene glycols (100 mg/kg)

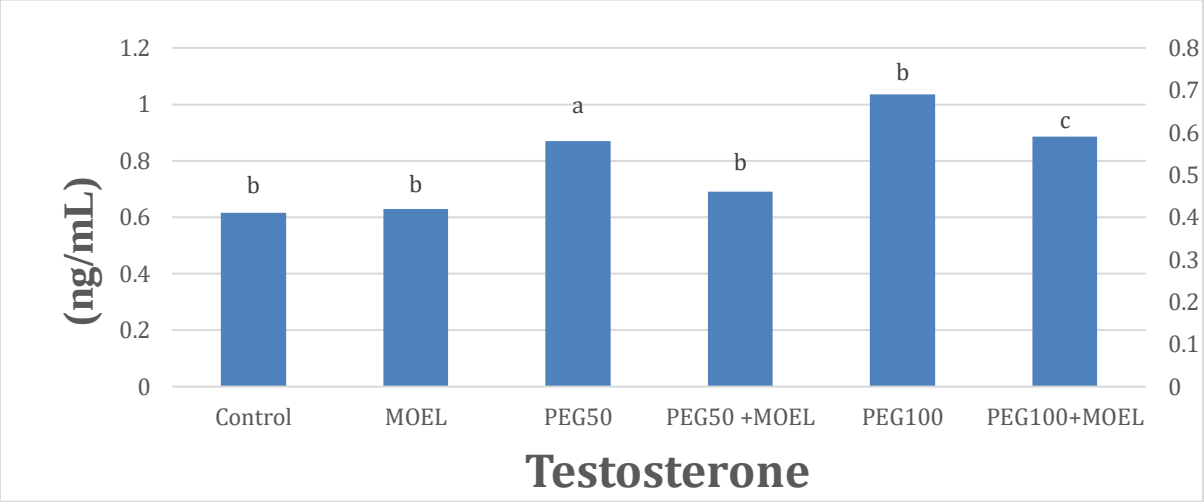

Figure (7): Effects of PEG and *MOLE* on testosterone in blood plasma of male rats

Conclusion

In conclusion, Exposure to polyethylene glycols induced reproductive toxicity via deteriorations at different levels of semen characteristics, induction of lipid peroxidation, depletion of the antioxidant activity, and oxidative harm by upsetting the equilibrium between reactive oxygen species (ROS) Regarding the male rats' antioxidant defense system, *Moringa oleifera leave extract* reduced the reproductive toxicity induced by PEG. Also, MOLE plays an important part in neutralizing ROS and protecting the tissues from oxidative harm brought on by PEG.

Conflicts of Interest:

The authors declare no conflict of interest.

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