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Original Article

Hepatotoxicity of Polyethylene Glycol and Possible Protection Using *Moringa Oleifera* Leaves Extract (MOLE)

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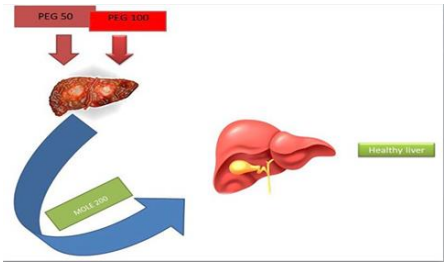
ABSTRACT

Polyethylene glycol (PEG) was used for a wide range of medical and biological applications. The health problems that PEG can cause is the conversion of alcohol dehydrogenase by metabolic oxidation into oxalate. *Moringa oleifera* contains about 46 antioxidant compounds such as β-carotene and various phenolics. Previous studies did not report enough data on the hepatotoxicity of polyethylene glycol and the protective role of *Moringa oleifera*. Therefore, the current study was conducted to address this affair. Male rats were split for six set (six each group): control group 1, MOLE (200 mg/ kg) group 2, PEG (50 mg/kg) group 3, MOLE plus PEG (50 mg/kg) group 4, PEG (100 mg/kg) group 5, and MOLE plus PEG (100 mg/kg) group 6. Rats administered orally daily for 45 days. The obtained results showed that treatment with both doses of PEG caused significant increase in DNA breakages, TNF-α, IL-6, TBARS, and NOx comparison to group 1. While, both doses of PEG caused significant suppressed expression of PGC-1α and mtTFA, the P53 level, catalase, GR, and GSH were decline, as compared with group 1. It was concluded that the co-supplementation with MOLE caused significant hepatoprotection against PEG-induced liver toxicity at all levels.

GRAPHICAL ABSTRACT



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Introduction

Polyethylene glycols (PEG) are polyether compounds which are widely used as additives in food, pharmaceuticals, and cosmetics [1]. Polyethylene glycol (PEG), also known as macrogol, is a polyether consisting of ethoxy units derived from the ring-opening polymerization of ethylene oxide [2]. PEG may reach sewage systems due to its use in industry [3]. It contains a huge amount of oxygen atoms and hydroxyl groups, and both can form hydrogen bonds [4]. The metabolism process of PEG involved successive oxidation via alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). This series of sequential reactions can be converted into the toxic receptors. The polyethylene glycol metabolites produce the renal destruction via mechanisms similar to those involved in the renal failure associated with ethylene glycol poisoning [5].

Moringa oleifera Lam, a fast-growing tree, was originated from India [6]. Various parts of *Moringa* included the paramount minerals and considered as a perfect source of protein and vitamins, different phenols, and act as antitumor, antioxidant, and hepatoprotective [7]. It leaves considered as a potential origin of natural antioxidants and the extracts capable of scavenging peroxyl and superoxyl radicals [8]. Therefore, the present study investigated the potential activity of MOLE as an antioxidant agent against the hepatotoxicity induced by PEG in male rats.

Materials and Methods

Tested compounds

Polyethylene glycol 1500 (PEG, purity 99.9 %) was purchased from Central Drug House Ltd., New Delhi, India. *Moringa oleifera* leaves extract (MOLE) was purchased from National Research Center, Dokki, Cairo, Egypt.

Animals and experimental groups

Wister male rats were used in the current study. Animals were obtained from Faculty of Medicine, Alexandria University, Alexandria, Egypt. This study was confirmed by Animal Care Committee and met all guide lines for its use (Institutional Animal Care and use Committee; ALEXU- IACUC). Animals were housed in a stainless-steel wire cages, maintaining a basic diet (food and water adlibitum) at a suitable and airtight ambience (temperature of 25 ± 5 °C, 50-70% humidity). Rats were divided into six equal groups (n = 6), and then the animals were kept for 14 days of adaptation. Group 1, control in which healthy untreated rats; group 2, MOLE 200 mg/kg; group 3, PEG 50 mg/kg; group 4, MOLE + PEG 50 mg/kg; group 5, PEG 100 mg/kg; and group 6, MOLE + PEG 100 mg/kg. Doses were given daily for 45 consecutive days. The selected doses of polyethylene glycol and *Moringa oleifera* leaves extract were based on Diab *et al.* and Jaiswal *et al.* [9, 10], respectively.

Blood samples collection and tissue preparation

After 45 days have ended, the rats were anesthetized with isoflurane and sacrificed. Sample of blood were collected in test tubes containing heparin as an anticoagulant. Plasma was separated from the blood by centrifuging at 860×g for 20 min and preserved at -80 °C for analysis. The liver was instantly removed and washed with the cold saline solution to carefully remove the adhered fat connective tissues. Separately, part of the liver was homogenized (10%, w/v) in the ice-cold sucrose buffer (0.25 M) in a Potter–Elvehjem type homogenizer, and then the homogenates were centrifuged at 10,000×g for 20 min at 4 °C. To pellet the cell debris, the supernatant was collected and saved at -80 °C for the determination of the rest of parameters.

Body and organs weights

The initial and final weights of the rats were recorded. Likewise, the weights of the livers were recorded instantly after their removal and dried on tissue papers.

Quantitative analysis of hepatic gene expression of mitochondrial transcription factor A (mtTFA) and peroxisome proliferator activator receptor gamma-coactivator 1α (PGC-1α) using RT-PCR

The quantitative expression analysis of PGC-1α and mtTFA in liver tissue were performed using for the relative quantitative determination of the gene expression of mtTFA [11] and PGC-1α [12] at mRNA level according to the manufacturer instructions. The primes sequences were used as follow: PGC-1α; F-5-AAACTTGCTAGCGGTCCTCA-3, and R- 5-TGGCTGGTGCCAGTAAGAG-3, mtTFA; F-5-CCTTCGATTTTCCACAGAACA-3, R-5-GCTCACAGCTTCTTTGTATGCTT-3, and GAPDH; F-5'-GGGTGTGAACCACGAGAAATA-3' and R-5' AGTTGTCATGGATGACCTTGG3'.

Assay of DNA breakages

The DNA breakages, as an indicator of cell death, were assayed according to the method of Wu *et al.* [13].

Enzyme linked immunosorbent assay (ELISA)

The homogenates of liver tissues were used for the determination of tumor necrosis factor-alpha (TNF-α; cat. no. ab100785), interleukin‐6 (IL‐6; cat. no. ab100772) and p53 (cat. no. ELR‐p53‐1; RayBiotech, Inc.), by using respective ELISA kits (Abcam) according to the manufacturer instructions.

Markers of oxidative stress

The TBARS level was determined according to Draper and Hadley [14]. Nitric oxide (NOx) gives nitrites and nitrates in the deproteinized samples; the Griess was used to determine the concentrations of the final products, after that reduction of nitrate to nitrite was occurred. The diazotization removal was carried out and the NOx level was determined from the slope of the standard curve constructed using a serial concentration of sodium nitrite according to Montgomery and Dymock [15].

Reduced glutathione (GSH), glutathione -S-transferase (GST; EC 2.5.1.1.18), glutathione peroxidase (GPx; EC 1.1.1.9), and glutathione reductase (GR; EC 1.6.4.2) were determined according to the described methods by Ellman, Habig *et al.*, Pagila and Valantin, and Panfili *et al.* [16-19], respectively. Kits were purchased from Bio diagnostic, Egypt.

Biochemical parameters

Plasma total protein (TP) concentration was measured according to Armstrong and Carr [20]. The total, direct, and indirect bilirubin concentrations were measured according to the method described by Price [21].

Aspartate aminotransferase (AST) (AST; EC 2.6.1.1) and alanine aminotransferase (ALT) (ALT; EC 2.6.1.2) were measured by the method of Reitman and Frankel (1957). Alkaline phosphatase (AlP) (AlP; EC 3.1.3.1), acid phosphatase (AcP) (AcP; EC 3.1.3.2), and gamma-glutamyltransferase (γ-GT) (γ-GT; EC 2.3.2.2) were measured according to the described methods by Belfield and Goldberg, Daniel *et al.*, and Persijin, and Van der Slike [22-24], respectively.

Catalase (CAT; EC 1.11.1.6) was determined according to the method described by Sinha [25]. Superoxide dismutase (SOD, EC 1.15.1.1) was assayed according to the method described by Nishikimi [26].

Drug metabolizing enzymes

The total hepatic content of the cytochrome b_5 and cytochrome P_{450} were estimated by the method of Omura [27]. The activity of NADPH-cytochrome C- reductase was assayed according to the method of Williams and Kamin [28], the activity of amidopyrine N-demethylase was measured according to Nash [29] and the activity of aniline 4-hydroxylase was measured according to the method described by Kato and Gillette [30].

Statistical analysis

Mean and standard error values were determined for all the parameters and the results were expressed as mean ± standard error. The data were analyzed using a one-way analysis of variance (ANOVA) followed by Duncan multiple comparison.

Percentage of change = (mean of treatment – mean of control) / mean of control×100

Results and Discussion

The obtained data showed that treatment with PEG of both doses and MOLE caused insignificant effects on body (Figure 1) and liver weights (data are not shown). A low dose of PEG caused an insignificant effect on the hepatic expression PGC-1α, while the high dose caused significant suppression, as compared with the control group. The PEG treated rats showed marked suppression of the expression of mtTFA at all doses, as compared with control. Moreover, the co-treatment of PEG plus MOLE substantially increases PGC-1α and mtTFA in liver tissue, as compared with PEG treated rats. The DNA breakages significantly increased in 50 and 100 mg/kg of PEG when compared with the control group. Meanwhile, MOLE substantially decreased the DNA breakages in the co-treatment group of 50 and 100 mg/kg of PEG, as compared with PEG (50 and 100 mg/kg) alone (Table 1 and Figure 2).

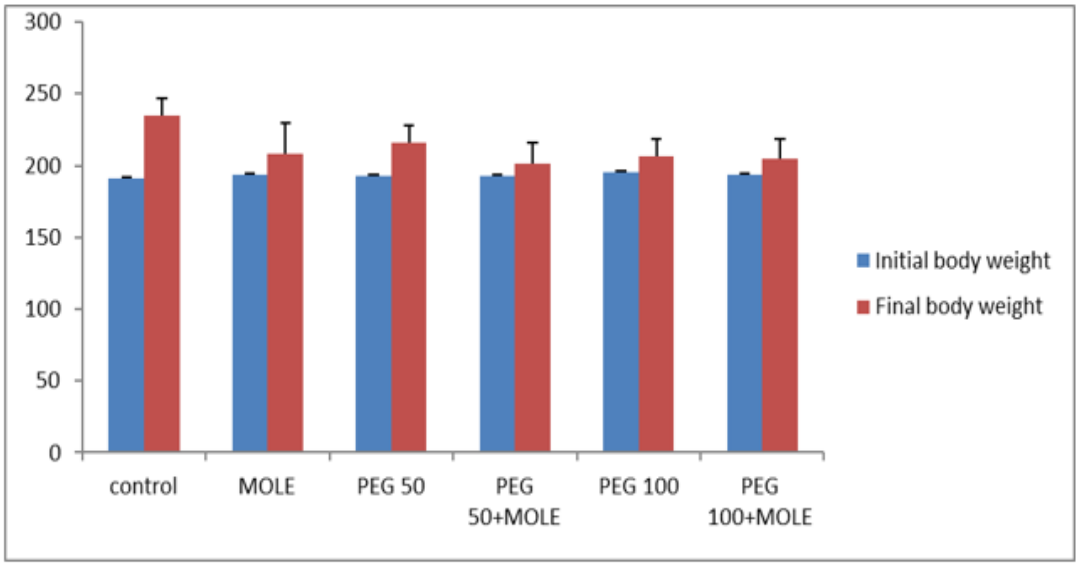

Figure 1: Initial and final body weight of male rats

Table 1: The effect of polyethylene glycol (PEG) and *Moringa oleifera* leaves extract (MOLE) on liver gene expressions of mitochondrial transcription factor A (mtTFA), gene expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), and DNA breakages of male rats

Experimental Groups	Parameter		
	mtTFA (Fold change)	(PGC-1α) (Fold change)	DNA breakages %
Control	1.0±0.27^{ab}	1.0±0.13^{ab}	13.4±0.53^{c}
MOLE	1.2±0.03^{ab} (22%)	1.4±0.13^{a} (42%)	12.7±0.87^{c} (-5.05%)
PEG (50 mg/kg)	0.4±0.04^{c} (-59%)	0.7±0.08^{b} (-22%)	24.3±1.37^{ab} (980.45%)
PEG (50 mg/kg) +MOLE	1.4±0.16^{a} (49%)	1.2±0.11^{a} (28%)	15.8±0.96^{c} (17.89%)
PEG (100 mg/kg)	0.3±0.07^{c} (-62%)	0.2±0.02^{c} (-72%)	27.3±1.83^{a} (102.89%)
PEG (100 mg/kg) +MOLE	0.7±0.07^{bc} (-27%)	0.8±0.10^{b} (-20%)	22.0±0.67^{b} (63.99%)

The results are expressed as (Mean ± SE, n=6)

^{abc} *Mean values within a column not sharing common superscript letters were significantly different, p< 0.05.*

The number between parentheses is the percentage of change from control value

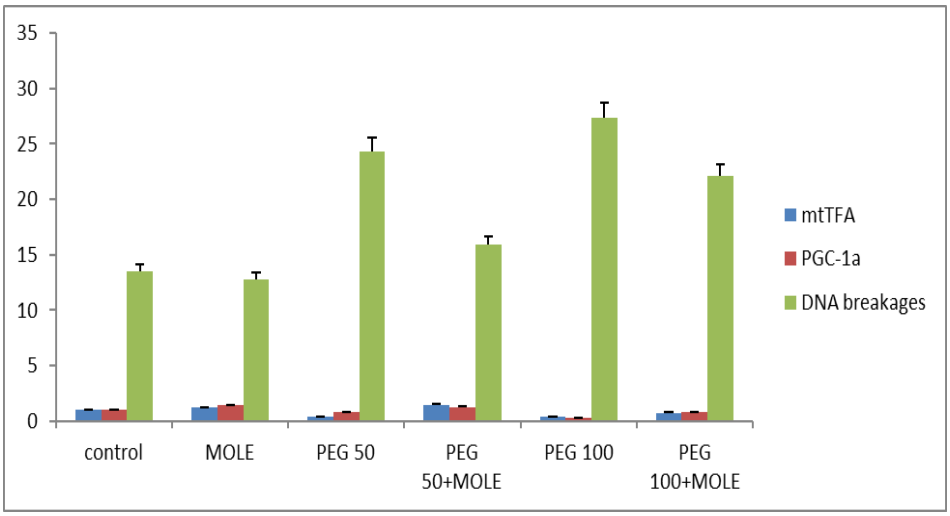

Figure 2: The effect of polyethylene glycol (PEG) and *Moringa oleifera* leaves extract (MOLE) on liver gene expressions of mitochondrial transcription factor A (mtTFA), gene expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), and DNA breakages of male rats

Results presented in Table 2 and Figure 3 showed that TNFα and IL-6 significantly increased in rats treated with PEG alone, as compared with the control group. Meanwhile, the treatment of rats with MOLE significantly decreased TNFα and IL-6 of groups treated with PEG 50 + MOLE and PEG 100 + MOLE, as compared with PEG 50 and PEG 100, respectively. On the other hand, P53 significantly decreased in rats treated with PEG alone, as compared with the control group. Meanwhile, P53 significantly increased in the group PEG 50 + MOLE and PEG 100 + MOLE, as compared with PEG 50 and PEG 100.

Table 2: The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on liver expression of tumor necrosis factors α (TNFα), interleukin 6 (IL-6), and expression of tumor suppressor P53 (P53) of male rats

Experimental Groups	Parameter		
	TNF-α (pg/mg protein)	IL-6 (pg/mg protein)	P53 (pg/mg protein)
Control	3.57±0.38^{d}	1.08±0.03^{d}	4.10±0.05^{ab}
MOLE	4.00±0.48^{d} (12.04%)	1.27±0.05^{d} (18.13%)	4.14±0.12^{a} (0.87%)
PEG (50 mg/kg)	39.9±1.86^{b} (1018.76%)	21.8±1.21^{b} (1924.97%)	3.64±0.13^{c} (-11.20%)
PEG (50 mg/kg) + MOLE	27.9±1.77^{c} (684.03%)	11.0±0.48^{c} (918.50%)	4.04±0.04^{ab} (-1.51%)
PEG (100 mg/kg)	60.0±1.58^{a} (1582.35%)	29.6±0.80^{a} (2638.20%)	3.5±0.14^{c} (-13.49%)
PEG (100 mg/kg) + MOLE	38.4±2.92^{b} (977.87%)	20.6±0.69^{b} (1806.56%)	3.7±0.05^{bc} (-7.57%)

The results are expressed as (Mean ± SE, n=6)

^{abc} *Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. The number between parentheses is the percentage of change from control value*

The obtained data showed that cytochrom b5 and P450 significantly increased and substantially decreased in doses of 50 and 100 of PEG, respectively, as compared with the control group. N-demethylase and NADPH cytochrome C-reductase substantially increased in the rats treated with both doses of PEG at doses of (50 and 100 mg/kg), as compared with the control group. Meanwhile, treatment with MOLE significantly decreased these enzymes in the co-treatment group at doses of 50 and 100 mg/kg of PEG in comparison to PEG treated alone. Aniline 4-hydroxylase significantly increased and substantially decreased in rats treated with PEG

alone, as compared with the control group. However, co-administration with MOLE significantly inhibited this enzyme compared with PEG treated. Cytochrome b5 (nmol cytochrome/mg protein), Cytochrome p450 (nmol cytochrom/mg protein), Amidopyrine N-demethylase (mol/min/mg protein), Aniline 4-hydroxylase (mol/min/mg protein) and NADPH cytochrome C-reductase (mol cytochrome C reductase/mg protein/min) (Table 3 and Figure 4).

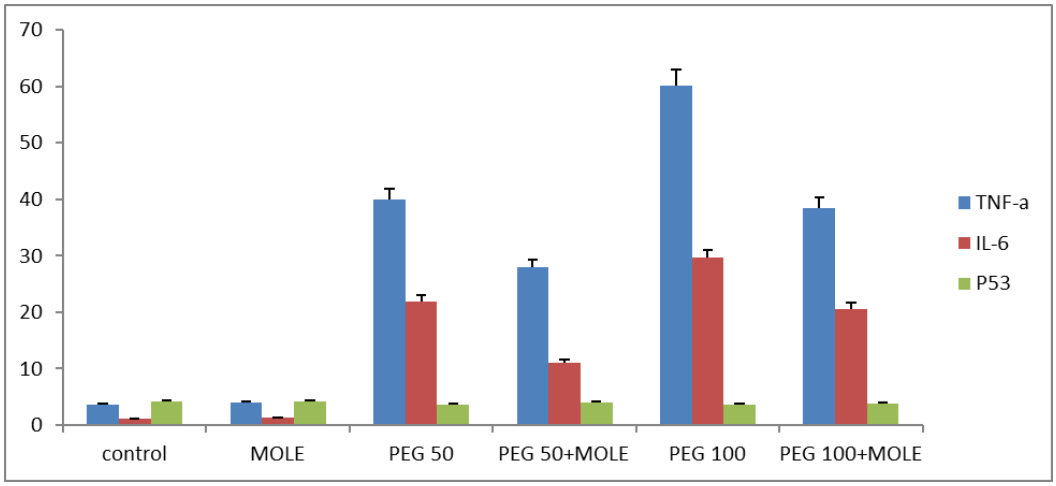

Figure 3: The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on liver expression of tumor necrosis factors α (TNFα), interleukin 6 (IL-6), and expression of tumor suppressor P53 (P53) of male rats

Table 3. The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on hepatic cytochrome b_5, cytochrome P_{450}, amidopyrine N-demethylase, aniline 4-hydroxylase, and NADPH cytochrome C-reductase of male rats

Experimental Groups	Parameter				
	Cytochrome b_5	Cytochrome p_{450}	Amidopyrine N-demethylase	Aniline 4-hydroxylase	NADPH cytochrome C- reductase
Control	0.3±0.01^{b}	0.6±0.05^{cd}	0.1±0.007^{d}	0.2±0.005^{b}	2.5±0.11^{d}
MOLE	0.7±0.04^{a} (106.08%)	1.2±0.04^{b} (81.42%)	0.3±0.008^{ab} (87.95%)	0.2±0.005^{b} (1.04%)	2.4±0.10^{d} (-4.53%)
PEG (50 mg/kg)	0.7±0.03^{a} (103.96%)	1.4±0.09^{a} (104.20%)	0.3±0.01^{a} (92.16%)	0.3±0.008^{a} (13.98%)	5.4±0.08^{b} (111.92%)
PEG (50 mg/kg) + MOLE	0.3±0.01^{b} (5.55%)	0.8±0.04^{c} (21.48%)	0.2±0.007^{c} (43.97%)	0.2±0.01^{c} (-20.27%)	4.4±0.08^{c} (74.11%)
PEG (100 mg/kg)	0.2±0.003^{c} (-23.54%)	0.5±0.005^{de} (-14.80%)	0.3±0.007^{a} (95.18%)	0.2±0.01^{c} (-17.48%)	6.2±0.07^{a} (143.99%)
PEG (100 mg/kg) + MOLE	0.2±0.009^{c} (-28.83%)	0.5±0.01^{e} (-24.96%)	0.2±0.01^{b} (72.89%)	0.3±0.007^{a} (15.73%)	1.9±0.21^{e} (-22.25%)

The results are expressed as (Mean ± SE, n=6).

^{abc} *Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. The number between parentheses is the percentage of change from control value.*

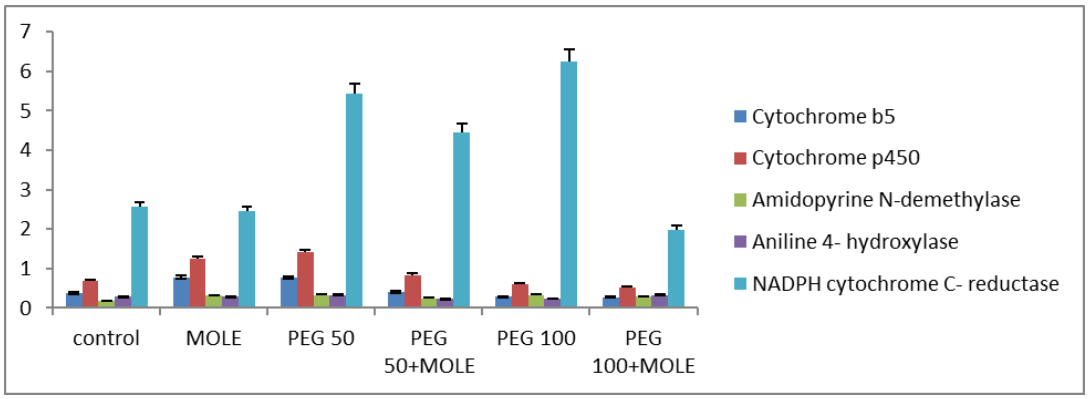

Figure 4: The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on hepatic cytochrome b_5, cytochrome P_{450}, amidopyrine N-demethylase, aniline 4-hydroxylase, and NADPH cytochrome C-reductase of male rats

It was observed that GR, GST, GPx, and GSH significantly decreased in animals treated with PEG alone in a comparison with the control group. Meanwhile, the MOLE administration significantly increased GR, GPx, and GST in the groups of PEG50 + MOLE and insignificantly changed GSH, as compared with PEG alone. However, GR significantly increased in animals treated with PEG 100 + MOLE, and GPx and GST significantly decreased, while GSH significantly changes, as compared with PEG, alone. The result showed that SOD in animals treated with PEG at dose 50 mg/kg caused an insignificant change and at dose 100 mg/kg caused significantly increased, as compared with the control group. Meanwhile, treatment with MOLE significantly decreased in the co-treatment in comparison to PEG treated alone. On the other hand, catalase in both doses of PEG caused a significant decrease compared with the control group. Nevertheless, in groups treated with PEG 50 + MOLE caused a substantial decline compared with PEG 50 and significantly increased in PEG 100 + MOLE, as compared with PEG 100 mg/kg (Table 4 and Figure 5).

Table 4: The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on liver glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S transferase (GST) reduced glutathione (GSH), superoxide dismutase (SOD), and catalase of male rats

Experimental Groups	Parameter					
	GR (IU/mg protein)	GPx (IU/mg protein)	GST (IU/mg protein)	GSH (IU/mg protein)	SOD (IU/mg protein)	Catalase (IU/mg protein)
Control	75.9±1.25^{a}	141.1±3.1^{a}	5.3±0.23^{c}	1.4±0.06^{b}	133±17.51^{b}	261±45.51^{a}
MOLE	92.0±1.03^{a} (21.17%)	109.5±3.7^{b} (-22.39%)	8.3±0.57^{a} (55.47%)	1.6±0.06^{a} (12.03%)	136±10.47^{b} (2.24%)	227±112.4^{a} (-12.94%)
PEG (50 mg/kg)	49.9±2.89^{c} (-34.25%)	65.6±4.2^{d} (-53.45%)	4.0±0.13^{d} (-23.31%)	0.6±0.01^{e} (-56.15%)	132±34.63^{b} (-1.04%)	148±146.5^{c} (-43.01%)
PEG (50 mg/kg) + MOLE	58.8±1.43^{b} (-22.48%)	92.8±4.61^{c} (-34.21%)	5.4±0.13^{c} (1.61%)	0.5±0.01^{e} (-61.47%)	118±37.98^{c} (-11.36%)	111±42.95^{d} (-57.21%)
PEG (100 mg/kg)	25.2±0.58^{e} (-66.74%)	156.6±6.9^{a} (11.00%)	6.7±0.15^{b} (26.18%)	0.7±0.03^{d} (-45.57%)	144±4.62^{a} (7.84%)	135±51.5^{cd} (-48.10%)
PEG (100 mg/kg) + MOLE	43.8±0.80^{d} (-42.26%)	90.8±2.62^{c} (-35.64%)	3.9±0.12^{d} (-26.63%)	1.2±0.06^{c} (-10.78%)	136±21.05^{b} (1.79%)	187±53.7^{b} (-28.11%)

The results are expressed as (Mean ± SE, n=6).

^{abc} *Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. The number between parentheses is the percentage of change from control value*

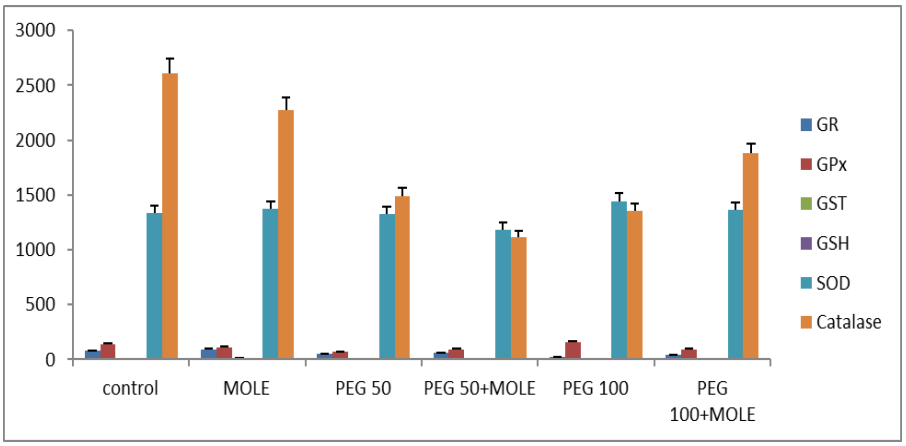

Figure 5: The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on liver glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S transferase (GST) reduced glutathione (GSH), superoxide dismutase (SOD), and catalase of male rats

Thiobarbituric acid reactive substances significantly increased in 50 and 100 mg/kg of PEG as compared with the control group. Meanwhile, treatment with MOLE significantly decreased in co-treatment at dose 50 and 100 mg/kg of PEG. On the other hand, NOx in animals treated with PEG at dose 50 mg/kg caused insignificant change and significantly increased in other dose of PEG that treated alone, as compared with control. Meanwhile, when treated with MOLE caused insignificant change with PEG at dose 50 mg/kg, while MOLE caused a substantial decline with PEG at dose 100 mg/kg compared with the group treated with PEG alone (Table 5 and Figure 6).

The results revealed that AST significantly increased in all groups administered with PEG and/or MOLE, as compared with the control group. ALT significantly decreased in PEG (50 and 100 mg/kg) and significantly increased in PEG 50 plus MOLE, γGT significantly increased in both doses of PEG treated alone compared with a control group and substantially decreased in PEG 100 mg/kg plus MOLE compared with PEG at dose 100 mg/kg alone. AlP and AcP significantly increased in both doses of the groups treated with PEG alone compared with a control group. Treatment with MOLE significantly decreased in AlP in PEG at dose of 100 mg/kg, and also AcP significantly decreased in PEG at dose of 50 and 100 mg/kg.

Table 5: The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on liver thiobarbituric acid reactive substances (TBARS) and nitric oxide of male rats

Experimental Groups	Parameter	
	TBARS (µmol/g tissue)	Nitric oxide (mU/mg protein)
Control	1.8±0.02^{cd}	179.1±6.87^{b}
MOLE	1.5±0.03^{d} (-14.38%)	170.8±4.81^{b} (-4.63%)
PEG (50 mg/kg)	2.1±0.03^{c} (21.83%)	170.6±6.93^{b} (-4.74%)
PEG (50 mg/kg) + MOLE	1.8±0.05^{cd} (5.11%)	191.2±6.37^{b} (6.75%)
PEG (100 mg/kg)	4.3±0.21^{a} (144.11%)	258.3±7.40^{a} (44.22%)
PEG (100 mg/kg) + MOLE	3.1±0.21^{b} (77.16%)	187.0±6.70^{b} (4.41%)

The results are expressed as (Mean ± SE, n=6).

^{abc} Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. The number between parentheses is the percentage of change from control value.

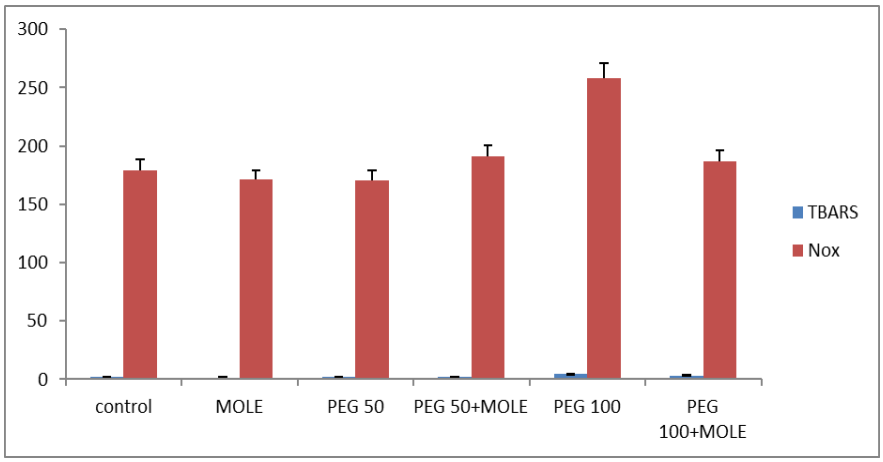

Figure 6: The effect of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on liver thiobarbituric acid reactive substances (TBARS) and nitric oxide of male rats

The present result showed the insignificant changes in both doses of PEG (50 and 100 mg/kg) in TP compared with the control group, and also in the group treated with MOLE in PEG at dose of 50 mg/kg but significantly increased in PEG at dose of 100 mg/kg compared with the rats treated with PEG (50 and 100 mg/kg) alone, respectively. The indirect bilirubin insignificantly increased in the groups treated with both doses of PEG compared with the control group. However, MOLE administration substantial increase in PEG at dose of 50 mg/kg and significantly decreased in PEG at dose of 100 mg/kg compared with the group treated with PEG 50 and 100 mg/kg alone. The direct bilirubin significantly increased in both doses of PEG treated alone compared with the control group, while the administration of MOLE significantly decreased (Table 6 and Figure 7).

Table 6: The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on plama total protein (TP), indirect and direct bilirubin in blood plasma of male rats

Experimental Groups	Parameter		
	TP (g/dL)	Indirect bilirubin (mg/dL)	Direct bilirubin (mg/dL)
Control	3.4 ± 0.08^{b}	0.499 ± 0.026^{cd}	0.371 ± 0.042^{cd}
MOLE	3.9 ± 0.15^{a} (15.52%)	0.6 ± 0.09^{bc} (34.26%)	0.5 ± 0.10^{bc} (59.02%)
PEG (50 mg/kg)	3.5 ± 0.12^{b} (3.70%)	0.4 ± 0.09^{d} (-16.83%)	1.2 ± 0.12^{a} (250.1%)
PEG (50 mg/kg) + MOLE	3.9 ± 0.07^{a} (13.67%)	0.7 ± 0.04^{bc} (40.68%)	0.6 ± 0.06^{b} (87.33%)
PEG (100 mg/kg)	3.7 ± 0.03^{b} (9.86%)	1.0 ± 0.08^{a} (105.6%)	0.8 ± 0.06^{b} (119.9%)
PEG (100 mg/kg) + MOLE	3.4 ± 0.13^{b} (0.17%)	0.7 ± 0.03^{b} (56.11%)	0.3 ± 0.02^{d} (-15.63%)

The results are expressed as (Mean ± SE, n=6).

^{abc} Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. The number between parentheses is the percentage of change from control value.

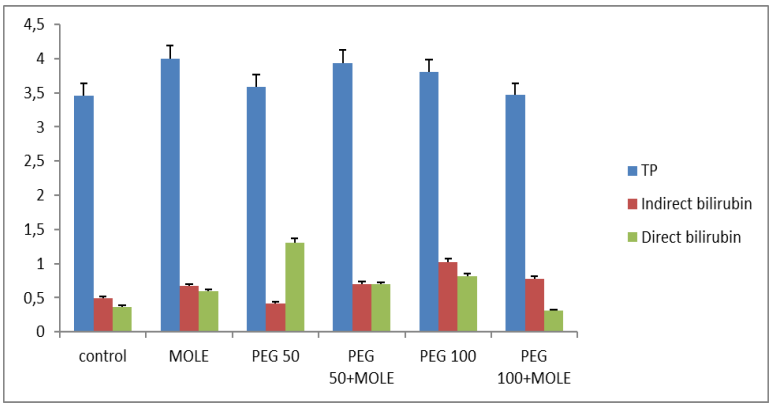

Figure 7: The effect of polyethylene glycols (PEG) and *Moringa oleifera* leaves extract (MOLE) on plama total protein (TP), indirect and direct bilirubin in blood plasma of male rats

The obtained results showed that the PEG and MOLE caused an insignificant effect on body and liver weights.

mTFA is a multi-functional transcription factor that is necessary for the maintenance of mtDNA integrity, replication, and transcription. The mtTFA expression is regulated by PGC-1 α, the main organizer of the biosynthesis of mitochondria. PGC-1α organizes various significant biological functions such as antioxidant defenses, cellular respiration, and adaptive thermogenesis through activating target genes (mtTFA and antioxidant enzymes).

Meyer *et al.* [31] reported that exposure to the environmental toxins leads to the mitochondrial dysfunction due to changes in the permeability of the mitochondrial membrane. In addition, this was what we have observed in the current results when rats were treated with PEG only, a decline of mitochondrial biogenic formation, reduce transcription, and replication of mtDNA, which ultimately causes a weakening of the mitochondrial function, this explains the decline in the mtTFA expression and PGC-1α in hepatic tissue.

The toxic substances cause the impaired activity of the electron transport chain by affecting the complex second and fourth of the respiratory chain as it leads to the electrons accumulation in the chain, and thus leads to transport and interaction with oxygen to form superoxide anion radical, this causes weakness in the mitochondrial activity [32] mentioned that the acute toxicity of ethylene glycol causes mitochondrial abnormalities and this is what we observed in the current results.

Diab *et al.* [9] found that DNA damage may be the result of free radical-mediated oxidative stress. Polyethylene glycol at dose of 100 and 200 mg/kg caused an increase in DNA breakages, this confirms the current results as it indicated that the PEG raise the levels of free radicals and decreased the antioxidant enzymes and glutathione in the liver.

Hatami *et al.* [33] demonstrated that PEG caused a decrease in ALT and an increase in AIP, while TP, γGT, albumin, and AST did not change.

The co-administration of PEG with MOLE resulted in the significant induction of hepatic expression of mtTFA and PGC-1α compared with the PEG treated rats at both doses 50 and 100 mg/kg. These ameliorative effects of MOLE may cause significant correction of mitochondrial biogenesis and functions. In line with the present results, Sosa-Gutiérrez *et al.* [34] documented that the curative of HepG2 cells with *Moringa oleifera* substantial increase in both mitochondrial complexes activities and protein content. In addition, Mansour *et al.* [35] approved that MOLE has a protective influence contra gamma radiation-induced hepatotoxicity through its free radical scavenging activity, enhancement of the antioxidant defense mechanism, amelioration of mitochondrial structure, and functions. The result showed that the MOLE presence with both doses of PEG reduced the levels of inflammatory factors (IL-6 and TNF-α). In line with these data, Mahajan *et al.* [36]

mentioned that *Moringa oleifera* declines the level of TNF-α and IL-6.

Chattopadhyay *et al.* [37] reported that *Moringa oleifera* protected from DNA breakages and these results are consistent with the present results.

Albrahim and Binobead [38] reported that *Moringa oleifera* increased alone or combined with the other compounds as GSH, GST, and GPx. These results are in line with the current results.

Hamed and El-Sayed [39] revealed that total protein and AST significantly increased and AIP decreased in the group exposed to pendimethalin and treated with MOLE.

Sharma *et al.* [40] studied the protective role of *Moringa oleifera*. The results showed that hepatic cytochrome b5, cytochrome P450 significantly decreased compared with the group treated with DMBA alone, which was agreed with the present results in cytochrom b5 and P450 in the group treated with PEG 50 + MOLE, as compared with PEG at dose 50 mg/kg.

Conclusion

From the present study, it can concluded that PEG induced hepatotoxicity in rats at different levels, oxidative DNA, and lipid damage, pro-inflammation impaired gene expression of PGC-1α and mtTFA and also changed drugs metabolizing enzymes. Likewise, the study clearly inducted the powerful hepato- protective effects of MOLE against PEG-induced toxicity.

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Authors' contributions

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Conflict of Interest

The author declared that they have no conflict of interest.

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