Original Article

Curtailment of Nephrotoxicity of Polyethylene Glycol via Moringa Oleifera Leaf Extract in Male Rats

Mokhtar Ibrahim Yousef[1], Emam Atiyah Ibadi[2*], Maher Abd El-Nabi Kamel[3], Sabah El-Banna[4]

[1]Department of Environmental studies, Institute of Graduate Studies and Research, Alexandria University, Egypt.

[2]Pharmacy Department, Al-Mustaqbal University College, Iraq.

[3]Department of Biochemistry, Medical Research Institute, Alexandria University, Egypt.

[4]Department of Environmental studies, Institute of Graduate Studies and Research, Alexandria University, Egypt

Abstract

Polyethylene glycols (PEG) are water-soluble chemicals composed of periodic ethylene glycol units, mostly used in industry and medicine. They have several chemical and physical features, which may lead to possible toxicity. Moringa oleifera is used as an antioxidant because of its ability to inhibition of protein denaturation and scavenge free radicals. Previous studies do not report enough data on the nephrotoxicity of polyethylene glycol and the protective role of Moringa oleifera leaf extract (MOLE). So, the current study was conducted to address this affair. Male rats were split for six set (six each group) (group 1, as control; group 2, MOLE; group 3, PEG 50 mg/kg; group 4, MOLE + PEG 50 mg/kg; group 5, PEG 100 mg/kg; group 6, MOLE + PEG 100 mg/kg). Rats were administered orally daily for 45 days. The obtained results showed that treatment with both doses of PEG caused a significant increase in IL- 6, TNF-α, NOx, TBARS, uric acid, and GST comparison to group 1. While, both doses of PEG significantly suppressed expression of PGC-1α, levels of p53, GR, GPx and GSH declined compared to group 1. The co-complementary with MOLE leads to safeguarding the kidney from the toxicity induced by PEG at all levels.

Key words: Polyethylene glycol; Moringa oleifera; Nephrotoxicity; Tumor necrosis factors α; Gene expressions of mitochondrial transcription factor A.

Abbreviations: IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha; NOx, nitric oxide.

Introduction

Polyethylene glycol (PEG) refer to polymers of ethylene oxide [HO–(CH2–CH2–O)n–H], may exist in liquid or low-melting solid condition based on their molecular weights (Castanho et al., 2009). FDA agree using PEG for medical fields as it was used in topical and anti-microbial cream, and its use could lead to reach to the circulatory system, and thus many lead to an increase in osmolality in the blood, calcium and kidney failure (Cadnapaphornchai et al., 1981; Herold et al., 1989; Pasut & Veronese, 2010). The German Cosmetic, Toiletry, Perfumery and Detergent Association explained many impurities when using PEG in cosmetics for example (dioxin, ethylene, ethylene oxide and heavy metals) (Fruijtier-Pölloth, 2005).

Ethylene glycol itself is not lethally toxic, the metabolic oxidation product of ethylene glycol, oxalic acid, is the lethal agent. Ethylene glycol oxidation and conversion to oxalate, which in turn ultimately produces an amino acid, which can cause kidney failure due to oxalate deposits in the renal tubes. Moringa oleifera Lam. is one of the most famous ancient if it is possible to prevent or reduce PEG oxidation, to prevent or reduce PEG toxicity (Achappa et al., 2019). So, the frequent exposure to a high dose of PEG shows toxic activity and causes tissue changes in kidney of mice, because of that, the world health organization (WHO) has determined that Quantity daily allowed 10 mg/kg from PEG. Therefore, awareness should be paid to the random use of this, compared to lower the risks that affect human health (WHO World Health Organisation, 1980; Diab et al., 2012).

Address for correspondence: Emam Atiyah Ibadi
Pharmacy Department, Al-Mustaqbal University College, Iraq.
Email: imam@uomus.edu.iq

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trees because of its medicinal and nutritional characteristic. Leaves, seeds, flowers and bark included a set of chemicals with biological effects (Ray et al., 2015). Antioxidant polyphenolic analysis of the leaves indicated the presence of several phenolic acids. Also the ethanolic extract appears elevated phenolic content, free radical scavenging capacity (Luqman et al., 2012; Verma et al., 2009).

Antioxidants very important for curb free radicals, so that safeguard body from contagion and degenerative illness. The aqueous extract of Moringa oleifera have powerful scavenging impact on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical, superoxide, nitric oxide radical and repression of lipid peroxidation and protect form the oxidative effects (Sreelatha & Padma, 2009).

No deaths or toxic signs were observed at dose 400, 800, 1600 and 2000 mg/kg (Adedapo et al., 2009). Researchers have confirmed that the consumption of Moringa oleifera leaves is safe up to 2000 mg/kg (Stohs & Hartman, 2015).

Material and research 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 - Egypt - Cairo – Dokki.

Animals Experimental design.

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 ad libitum) suitable and airtight ambience (temperature of 25 ± 5 °C, 50-70% humidity). 14 days of adaptation. Rats were equally divided into six equal groups (n = 6). 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; 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 the (Diab et al., 2012) and (Jaiswal et al., 2009), respectively.

Blood Samples Collection and Tissue Preparation

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

Body and Kidneys Weights

The weights of the rat's primary and final were recorded. Also, the weights of the kidneys 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) usingqRT-PCR

Quantitative expression analysis of PGC-1α and mtTFA in kidney tissue was performed using for the relative quantitative determination of the gene expression of mtTFA (Piantadosi & Suliman, 2006) and PGC-1α (Li et al., 2011) at mRNA level according to the manufacturer instructions. The primes sequences used: PGC-1α; F- 5-AAACTTGCTAGCGGTCCTCA-3,and R- 5-TGGCTGGTGCCAGTAAGAG-3, mtTFA; F- 5-CCTTCGATTTTCCACAGAACA-3,and R- 5-GCTCACAGCTTCTTTGTATGCTT-3 and GAPDH; F- 5'-GGGTGTGAACCACGAGAAATA-3' and R- 5'-AGTTGTCATGGATGACCTTGG3'.

Enzyme linked immunosorbent assay (ELISA)

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

Markers of Oxidative Stress

The level of TBARS was determined according to (Draper & Hadley, 1990) 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. Diazotization removal requires sulfuric acid with nitrite ions with product coupling with a diamine to lead to a pink metabolite that is measured at 540 nm. The level of NOx was determined from the slope of the standard curve constructed using serial concentration of sodium nitrite (Awooda, 2013).

Tissues biochemical analysis

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 methods described by (Ellman, 1959; Habig et al., 18974; Paglia and Valantin, 1967; Panfili et al., 1991), respectively. Kits were purcased from Bio diagnostic, Egypt.

Blood biochemical analysis

Urea was determined according to (Patton and Crouch, 1977), Creatinine was determined according to (Schirmeister et al., 1964) and Uric acid was determined according to (Barham & Trinder, 1972) by the kits obtained from Bio diagnostic, Egypt.

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

The obtained data showed that treatment with PEG of both doses and MOLE caused insignificant effects on body and kidneys weights (data not shown).

The present results showed that, the gene expression of PGC-1αand mtTFA are significantly decreased in PEG 50 and PEG 100 in comparison to the control group, and lowest expression in rats exposed to 100 mg/kg PEG. The co-treatment of rats with MOLE significantly increased PGC-1α and mtTFA. MOLE completely normalized the expression in the rats treated with 50 mg/kg PEG and significantly up-regulate the expression of both genes in the rats treated with 100 mg/kg PEG (Table 1 and Fig 1).

Table 1. Effects of polyethylene glycol (PEG) and Moeinga oleifera leaves extract (MOLE) on kidney gene expressions of mitochondrial transcription factor A (mtTFA) and gene expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) of male rats.

Experimental Groups	Parameter	
	PGC-1α (Fold change)	mtTFA (Fold change)
Control	1.00±0.093ab	1.00±0.121b
MOLE	1.30±0.075a (30%)	1.26±0.052ab (26%)
PEG (50 mg/kg)	0.82±0.026b (-18%)	0.83±0.096bc (-17%)
PEG (50 mg/kg) + MOLE	1.00±0.047ab (0%)	1.60±0.161a (60%)
PEG (100 mg/kg)	0.30±0.054c (-70%)	0.49±0.028c (-51%)
PEG (100 mg/kg) + MOLE	0.79±0.122b (-21%)	0.92±0.092bc (-8%)

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

[abc]Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. Number between parentheses is the percentage of change from control value.

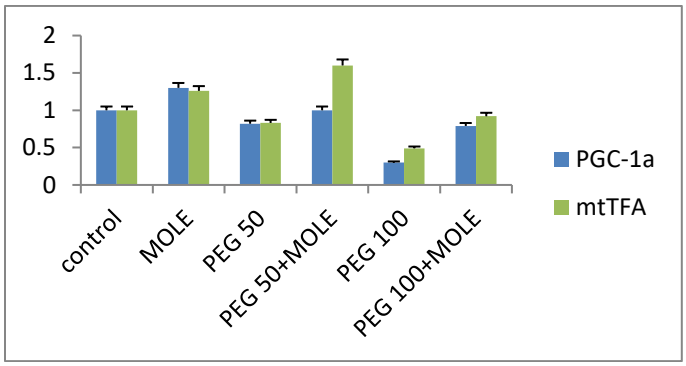

Fig 1: Effects of polyethylene glycol (PEG) and Moeinga oleifera leaves extract (MOLE) on kidney gene expressions of mitochondrial transcription factor A (mtTFA) and gene expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) of male rats.

TNFα and IL-6 significantly increased in PEG 50 and PEG 100 in comparison to the control group. Meanwhile, MOLE significantly decreased TNFα and IL-6 in PEG 50 + MOLE and PEG 100 + MOLE when compared to PEG 50 and PEG 100, respectively. However, P53 significantly decreased in PEG 50 and PEG 100 when compared to the control group. Meanwhile, P53 significantly increased in PEG 50 + MOLE and PEG 100 + MOLE when compared to PEG 50 and PEG 100, respectively (Table 2 and Fig 2).

Table 2. Effects of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on kidney 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	4.920±0.463d	1.117±0.025d	9.081±0.469b
MOLE	5.160±0.309d (4.87%)	0.978±0.071d (-12.44%)	10.44±0.441a (14.96%)
PEG (50 mg/kg)	26.36±1.436b (435.77%)	10.55±0.597b (844.49%)	6.377±0.591c (-29.77%)
PEG (50 mg/kg) + MOLE	10.21±0.392c (107.52%)	5.179±0.428c (363.65%)	9.348±0.370ab (2.94%)
PEG (100 mg/kg)	47.56±1.475a (866.66%)	23.45±1.805a (1999.37%)	4.910±0.276d (-45.93%)
PEG (100 mg/kg) + MOLE	28.48±1.055b (478.86%)	9.450±0.316b (746.01%)	8.652±0.423b (-4.72%)

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

[abc]Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. Number between parentheses is the percentage of change from control value.

Ethical, moral standards, non-disclosure agreement

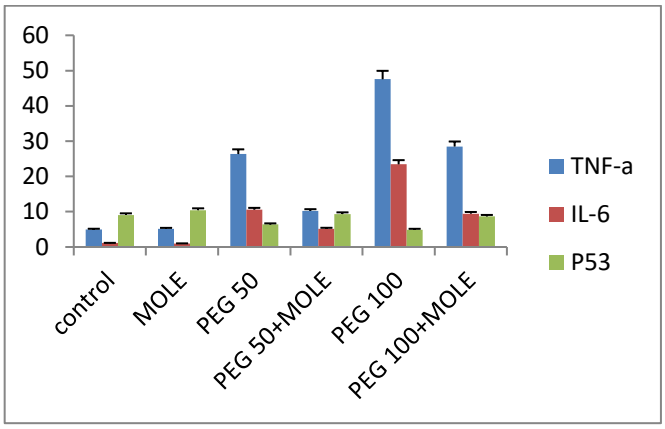

Figure 2. Effects of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on kidney expression of tumor necrosis factors α (TNFα), interleukin 6 (IL-6) and expression of tumor suppressor P53 (P53) of male rats.

GR, GPx and GSH significantly decreased in groups treated with PEG only in both doses comparison with the control group, in co-treatment observed significantly increased in both doses compared with groups treated with PEG only. While, the PEG caused significant increas in both doses in GST and significantly decreased in co-treatment (Table 3 and Fig 3).

The treatment of rats with PEG only in both doses caused significantly increase in the levels of TBARS and NOx compared with control groups and at the same time MOLE resulted in lower levels in co-treatment compared to groups treated with PEG only (Table 4 and Fig 4).

Table 3. Effects of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on kidney glutathione reductase (GR), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione-S transferase (GST) of male rats.

Experimental Groups	Parameter			
	GR (IU/mg protein)	GPx (IU/mg protein)	GSH (IU/mg protein)	GST (IU/mg protein)
Control	102.8±0.121a	819.8±15.49a	24.33±0.816a	4.979±0.162e
MOLE	103.0±2.161a (0.19%)	867.6±5.169a (5.83%)	20.26±0.286b (-16.72%)	7.166±0.130d (43.92%)
PEG (50 mg/kg)	65.55±1.818c (-36.23%)	403.1±16.70c (-50.82%)	14.06±0.520d (-42.21%)	19.98±0.316a (301.28%)
PEG (50 mg/kg) + MOLE	85.70±2.364b (-16.63%)	762.3±11.28b (-7.01%)	17.06±0.339c (-29.88%)	12.51±0.256b (151.25%)
PEG (100 mg/kg)	28.23±0.875d (-72.53%)	308.2±16.37d (-62.40%)	13.16±0.372d (-45.91%)	20.42±0.106a (310.12%)
PEG (100 mg/kg) + MOLE	32.77±1.153d (-68.12%)	391.4±22.31c (-52.25%)	15.99±0.869c (-34.27%)	8.978±0.130c (80.31%)

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

[abc]Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. Number between parentheses is the percentage of change from control value.

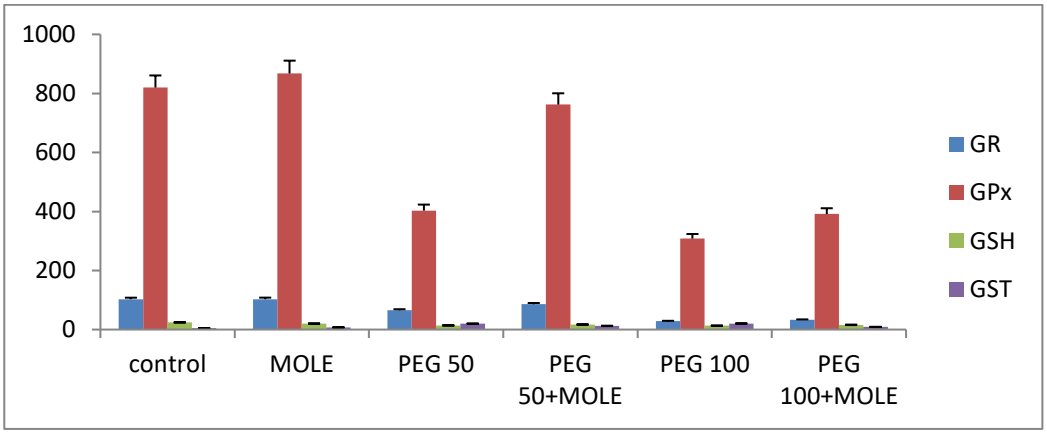

Figure 3. Effects of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on kidney glutathione reductase (GR), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione-S transferase (GST) of male rats.

Table 4. Effects of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on kidney thiobarbituric acid reactive substances (TBARS) and nitric oxide of male rats.

Experimental Groups	Parameter	
	TBARS(µmol/g tissue)	Nitric oxide (mU/mg protein)
Control	1.422±0.028d	67.22±2.443b
MOLE	1.057±0.056e (-25.66%)	64.61±1.360b (-3.88%)
PEG (50 mg/kg)	1.872±0.069b (31.64%)	70.29±1.282b (4.56%)
PEG (50 mg/kg) + MOLE	1.645±0.054c (15.68%)	46.66±1.830c (-30.58%)
PEG (100 mg/kg)	2.602±0.058a (82.98%)	79.78±3.247a (18.68%)
PEG (100 mg/kg) + MOLE	1.978±0.033b (39.09%)	64.05±4.252b (-4.71%)

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

[abc]Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. Number between parentheses is the percentage of change from control value.

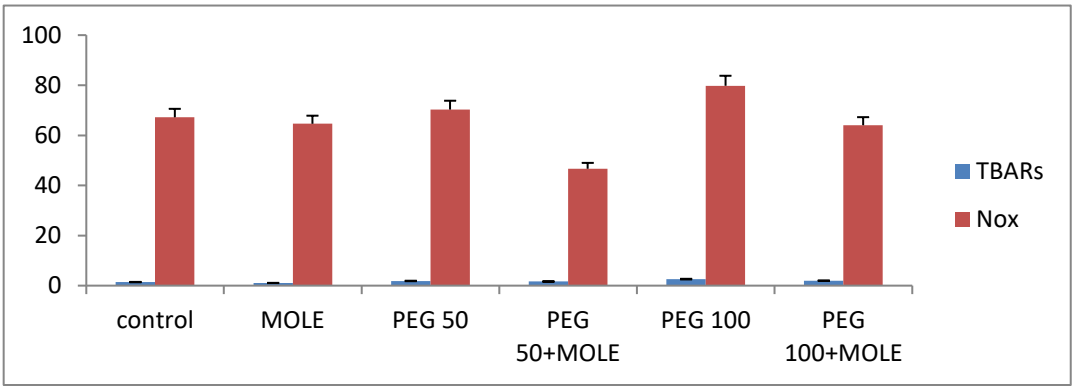

Figure 4. Effects of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on kidney thiobarbituric acid reactive substances (TBARS) and nitric oxide of male rats.

In the treatment group with PEG at dose (50 mg/kg) the results showed significantly increase in urea and uric acid

and decrease in creatinine, but at a dose 100 mg/kg it was a clear increase in both urea, creatinine and uric acid comparison with control group. In the co-treatment at dose (50 mg/kg) significantly increased in urea and creatinine is observed, and decrease in uric acid when comparison with the group treated with PEG only at dose (50 mg/kg). As for the co-treatment at dose (100 mg/kg) the results showed significantly increase in urea, decreased in creatinine and insignificantly change in uric acid compared with the group treated with PEG only at dose (100 mg/kg) (Table5 and Fig 5).

Table 5: Effects of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on kidney urea, creatinine and uric acid of male rats

Experimental groups	Parameter		
	Urea (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)
Control	24.14±0.685d	2.764±0.127ab	3.374±0.247c
MOLE	29.05±1.681d (20.33%)	1.714±0.111d (-38%)	3.388±0.107c (0.41%)
PEG50	29.59±0.771d (22.57%)	2.193±0.106c (-20.65%)	5.858±0.189a (73.62%)
PEG50+MOLE	50.42±2.854b (108.86%)	2.599±0.175b (-5.96%)	4.531±0.174b (34.29%)
PEG100	38.46±1.259c (59.32%)	3.133±0.127a (13.35%)	6.084±0.195a (80.32%)
PEG100+MOLE	58.57±3.451a (142.62%)	2.525±0.132bc (-8.64%)	5.865±0.080a (73.82%)

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

[abc]Mean values within a column not sharing common superscript letters were significantly different, p< 0.05. Number between parentheses is the percentage of change from control value.

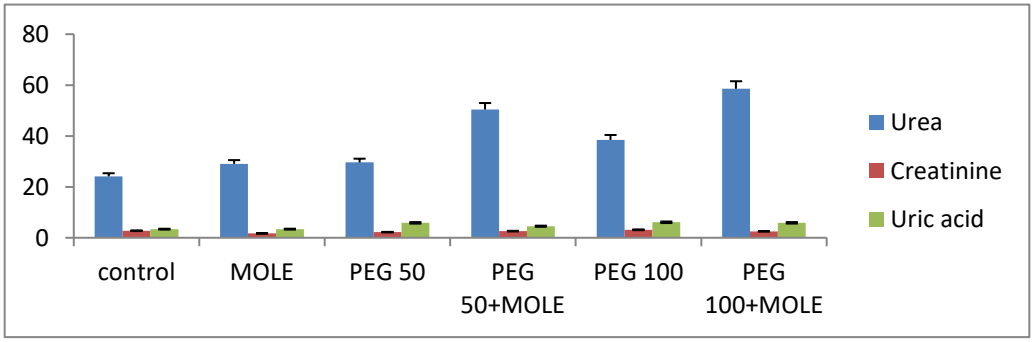

Figure 5. Effects of polyethylene glycols (PEG) and Moringa oleifera leaves extract (MOLE) on kidney urea, creatinine and uric acid of male rats.

Discussion

The results obtained showed that treatment with PEG in two doses and MOLE caused insignificant changes were observed in body and kidney weights and this is compatible with (Ueda et al., 2011; Osman et al., 2012).

The current study observed decline in the gene expression of PGC-1α and mtTFA in the groups treated with both doses of PEG. Studies have indicated that maintaining mitochondrial function is very important for the prevention of many diseases. Where (Wenz, 2011) explained that PGC-1α is the main organizer of biomechanics of mitochondria because it targets many cellular processes and thus affects the fate of the cell at multiple levels. (Toki et al., 2010) further explained that mtTFA is necessary for transcription and maintenance of mtDNA and works to protect DNA from oxidative stress.

Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are cytokines very important in the regulation of B-cell function where the last cells can produce them upon appropriate stimulation, when diseases related to B-cell function occur, increased production of these cytokines is observed (Rieckmann et al., 1997). (Popko et al., 2010) explained that IL-6 and TNF-α are the two main factors responsible for inflammatory conditions in the tissues. (Ridker et al., 2000; Spranger et al., 2003) indicated that IL-6 plays a central role in inflammation and tissue injuries and that high concentrations indicate the development of several diseases. In this present study, notice that the two doses of PEG heighten the inflammatory condition.

It protects p53 from DNA damage by coordinating a set of mechanisms early in its evolution metazoan, it stops the cell cycle to allow time for DNA repair and thus p53 performs multiple tasks in providing protection from the development of cancer by maintaining the stability of the genome. The loss of P53 contributes to a high rate of development of cancer (Williams & Schumacher, 2016). In the current study observed that the groups treated with PEG alone in both doses, caused minify p53 compared to control.

(Sies, 1999) Reminded that once the GSH formed in the liver, it participates in the reactions of oxidation and reduction and is transmitted through the membranes, especially the plasma membrane, which ultimately reaches the bloodstream to supply the other tissues, which is critical in the survival of aerobic cells. It functions as substrate for peroxidases and S-transferases and its importance as an extracellular radical scavenger (Bratoli and Sies, 1978; Kannan et al., 1996). It has a role in gene expression, apoptosis and signal transduction (Arrigo, 1999; Blackburn et al., 1999). In the current study, the ability to scavenging free radicals decreased in groups treated with PEG. (Pareta et al., 2011) Reported that GR decreased in group treated with ethylene glycol.

PEG caused high oxidative stress, and this is what we notice in a level TBARS and NOx in the groups treated with PEG alone.

Consistent with the results of the current study (Manjula et al., 2012), revealed the increase in the levels of urea, creatinine and uric acid in groups treated with ethylene glycol.

MOLE treatment with PEG in both doses resulted in an improvement in the gene expression of PGC-1α and mtTFA by increasing it compared with the groups treated with PEG alone. Studies indicated that improvement in gene expression leads to an improvement in mitochondrial function and protection of DNA from oxidative stress. (Gopalakrishnan et al., 2016) Explained that one of the mechanisms of fighting diabetes in Moringa oleifera is to protect the function of mitochondria.

MOLE in the co-treatment reduced the effect of PEG in TNF-α and IL-6 when compared with PEG alone, and that lead to alleviation the ratio of inflammation in line with these data, (AL-MALKI. A. & EL RABEY., 2015) that IL-6 significantly decreased when the rats treated with Moringa oleifera seeds powder. Increased P53 in co-treatment groups compared with group treated with PEG alone, as a rise P53 reduces the rate of cancer development.

The result showed that presence of MOLE with both doses of PEG increased the glutathione with high level of antioxidant.

(Hamed and El-Sayed, 2019) Revealed that MOLE caused decreased the level of creatinine and uric acid, which agree with the present results.

From the current study, we can conclude that treatment with PEG caused a decrease in gene expression and cancer cell resistance and at the same time increased levels of free radicals as well as levels of inflammation in renal cells. Whereas MOLE treated all the toxic effects of PEG.

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