

Amelioration of the nephrotoxic effect of potassium dichromate by whey protein and/or *Nigella sativa* oil in male albino rats

Samir A. E. Bashandy¹, Mohamed M. Amin^{1*}, Fatma A. Morsy², Salma A. El-Marasy¹

¹Department of Pharmacology, Medical Division, National Research Centre, 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt.

²Department of Pathology, National Research Centre, 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt.

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ABSTRACT

Even though whey protein complex derived from milk, is being touted as a functional food with a number of health benefits, so far its full mechanistic effect has not been deeply explicated, which is the target of this study. For this reason, we observed the 2 months protection influence of whey protein (100 & 200 mg/kg, p.ob.wt.) and/or oil of *Nigella sativa* (5ml/kg, p.ob.wt.) against intoxicated rats with single dose of potassium dichromate (30mg/Kg, I.P) at the end of protection period. On the biochemical level, whey protein and/or oil of *Nigella sativa* were changed the potassium dichromate renal toxicity consequences, where it showed a significant improvement in the creatinine, urea, protein, uric acid and sodium levels. While in the tissue level (Kidney), it revealed a significant enhancement in reduced glutathione (GSH), catalase and superoxide dismutase (SOD) enzymes as compared to nephrotoxic group. Moreover, whey protein and/or oil of *Nigella sativa* reduced the elevation of malondialdehyde (MDA) and inflammatory mediators like tumor necrotic factor-alpha (TNF- α) and nitric oxide (NO) induced by potassium dichromate and alleviation the histopathological changes caused by the intoxication. These results give a new vision into the hopeful mechanisms that modulated numerous factors induced renal injury.

INTRODUCTION

Individuals are exposed intentionally and unintentionally to a several of different chemicals that damage the kidney. As the number of medicines, natural compounds, industrial elements and environmental pollutants that cause nephrotoxicity has increased, the importance of this disease will grow up till the scientists discover a natural solution that can be used as a protective and preventive tool against kidney damage (Liu *et al.*, 2015). From these chemical and industrial nephrotoxic compounds is potassium dichromate, which is a potent oxidizing agent displaying a marked affinity, once reduced to trivalent chromium (Cr⁺³) by numerous cell metabolites, to

form several complexes with diverse biological ligands, including nucleic acids (Proietti *et al.*, 2005). Also, chromium induced the generation of Reactive Oxygen Species (ROS) that produce many toxic effects, including DNA destruction and lipid peroxidation that causing nephrotoxicity (Kelly *et al.*, 2006). Whey, a protein complex obtained from milk, including lactoferrin, betalactoglobulin, alpha-lactalbumin, glycomacropeptide, and immunoglobulins, exhibits a variety of immune-improving properties and the ability to act as an antioxidant and chelating agent for scavenging free radicals (Calvello *et al.*, 2016). Whey protein is also having a high percentage of sulfur-containing amino acids (cysteine and methionine), which boost immune task *via* intracellular conversion to glutathione.

Moreover, Lactoferrin, act as iron-binding glycoprotein, playing a key role as a non-enzymatic antioxidant (Calvello *et al.*, 2016). *Nigella sativa* seeds contains 36 -38% fixed oils, proteins, alkaloids and 0.4-2.5% essential oil.

* Corresponding Author

Mohamed M. Amin, Department of Pharmacology, Medical Division, National Research Centre, 33 EL Bohouth st. (former EL Tahrir st.), Dokki, Giza, Egypt. Email: mohamedmahmoud1983@gmail.com

Experimental studies have proved that *Nigella sativa* extract has a diversity of curative effects including hypertension, diabetes, renal injury, immune-modulative and liver protection. *Nigella sativa* can provide significant alleviation of the nephrotoxic effect of rats in relation to its free radical scavenging and antioxidant properties (Begum *et al.*, 2006). Finally, whey protein and the oil of *Nigella sativa* seems to be an important supplements in order to evaluate its capability to restoring renal injury accompanying inflammatory disorders, and complications.

MATERIAL AND METHODS

Drugs and chemicals

Whey protein was purchased from Davisco Food International Company, USA, while oil of *Nigella sativa* was purchased from Mepaco, Cairo, Egypt and potassium dichromate was obtained from Sigma Aldrich, USA. Whey protein was administered orally, which suspended in distilled water.

Animals

Adult male Wistar albino rats weighing 140-150 g were purchased from the National Research Centre Laboratory (Dokki, Giza, Egypt) and were housed in a standard polypropylene cages and kept under persistent environmental conditions with equal light-dark cycles. Rats were adapted for 1 week and were fed rat normal fat pellet diet and water ad libitum.

Ethics statement

This experiment was carried out in according to recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85-23, revised 1996) and under regulations of Animal Care and Use of National Research Centre in Egypt with ethical approval No. 15169. All surgery was performed under deep sodium pentobarbital anesthesia and all efforts were made to minimize suffering.

Induction of nephrotoxicity using potassium dichromate and experimental design

70 rats were divided into 7 groups (group=10 rats), the first one was received the vehicle and considered as normal control group. While, the second one treated with potassium dichromate only (30 mg/Kg Ip, single injection) (Mahmood *et al.*, 2010). Third group treated with 100mg/Kg whey protein (Low dose) (Eliwa *et al.*, 2014) orally administered daily for two months, and then challenged with potassium dichromate(30 mg/Kg Ip, single injection).

Fourth group orally administered low dose of whey protein plus oil of *Nigella sativa* (5ml/Kg, orally administered for two months (Develi *et al.*, 2014), then challenged with potassium dichromate (30 mg/Kg Ip, single injection). Fifth group orally administered 200 mg/Kg (high dose) (Eliwa *et al.*, 2014) whey protein only daily for two months, then challenged by potassium dichromate (30 mg/Kg Ip, single injection). Sixth group orally

administered high dose of whey protein plus oil of *Nigella sativa*, then challenged with potassium dichromate (30 mg/Kg Ip, single injection).

Seventh group administered orally oil of *Nigella sativa* only for two months, then challenged with potassium dichromate (30 mg/Kg Ip, single injection). The samples were collected 24 hours post potassium dichromate injection. Body weight of rats for each group was measured after the experiment.

Plasma collection for analysis

Blood was collected in heparinized tubes from the retro orbital plexus of veins under brief sodium pentobarbital anesthesia and was centrifuged (700×g, 4°C, 20 min) to separate plasma. Colorimetric kits were purchased from Salucea Company, Netherlands to estimate plasma urea, creatinine, uric acid, protein, and sodium.

Kidney Tissue Extract

After blood collection, rats were decapitated under a deep sodium pentobarbital anesthesia. Kidneys were separated out, washed, weighed and homogenized in phosphate buffer solution [PBS] [10%]. Tissue homogenate was centrifuged at 1500×g at 4°C for 15 minutes and the supernatant was collected and stored at -80°C for the direct assessment of parameters.

Assessment of renal oxidative stress parameters

Renal malondialdehyde (MDA), glutathione (GSH), catalase (CAT), super oxide dismutase (SOD) and nitric oxide (NO) were determined using colorimetric kits obtained from Bio-diagnostic, Egypt. ELISA technique was performed for the assessment of tumor necrosis alpha (TNF- α) was bought from R&D Systems, USA.

Histopathological examination of kidney

The kidney of different groups were removed and fixed in 10 % normal saline, 5um thick paraffin sections were stained with haematoxylin and eosin and investigated by light microscope (Shukla *et al.*, 2007).

Statistical Analysis

Values were stated as mean \pm S.E. of 8-10 rats and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc test estimated by SPSS software, version 21. The level of statistical significance was at $P < 0.05$.

RESULTS

Kidney function parameters

In this study, after intoxication of rats with potassium dichromate, rats showed a state of nephrotoxicity which was confirmed by a significant elevation of serum creatinine, urea, uric acid, proteins and sodium levels in comparison with normal

control group. Meanwhile, treated nephrotoxic groups with either whey protein (both doses) or/and *Nigella sativa* oil manifested a marked improvement in these parameters in relation with untreated nephrotoxic group (Table.1).

Physical parameters

Also, after intoxication of rats with potassium dichromate, rats presented a significant state of weight loss and an increase in kidney weight in comparison with normal control group. Surprisingly, all treated nephrotoxic groups showed a remarkable amelioration in body weight and kidney weight compared with untreated nephrotoxic group (Table. 2).

Oxidative stress parameters

Table 3 presented the significant decrease of antioxidant capacity of untreated nephrotoxic group reflected on the decline of

CAT, GSH and SOD and elevation of MDA in comparison with the normal control group. On the other side, treated nephrotoxic groups with either whey protein (both doses) or/and *Nigella sativa* oil manifested an excellent noticeable alteration in these parameters in relation with untreated nephrotoxic group.

Inflammatory parameters

Untreated nephrotoxic group showed a significant noticed increase in renal TNF- α , and NO levels compared with normal control group as shown in Figure 1 and 2.

Treated nephrotoxic groups elucidated a significant decrease in these parameters level in relation with untreated nephrotoxic group, but the effect of high dose of whey protein in combination with *Nigella sativa* oil was more predominate and significant than other treated nephrotoxic groups (Figure 1 and 2).

Table 1: Protective effect of whey protein, *Nigella sativa* oil and their combination on serum creatinine, urea, uric acid, proteins and sodium levels in potassium dichromate intoxicated rats.

Parameters	Control (vehicle)	Nephrotoxic (Pot.Di.Chr.)	Nephrotoxic (W prot. Low dose)	Nephrotoxic (W prot. Low dose + N.O.)	Nephrotoxic (W prot. High dose)	Nephrotoxic (W prot. high dose + N.O.)	Nephrotoxic (N.O.)
Creatinine (mg/dl)	0.48±0.03	1.48±0.07 [*]	0.87±0.04 [#]	0.79±0.06 [#]	0.62±0.05 [#]	0.65±0.05 [#]	0.8±0.06 [#]
Urea (mg/dl)	24.78±0.12	77.87±0.4 [*]	49.45±0.25 [#]	42.09±0.24 [#]	50.8±0.3 [#]	36.69±0.2 [#]	37.88±0.22 [#]
Uric acid (mg/dl)	5.16±0.09	6.43±0.11 [*]	4.15±0.08 [#]	4.01±0.07 [#]	5.11±0.1 [#]	4.9±0.09 [#]	5.05±0.12 [#]
Proteins (g/l)	6.99±0.1	8.06±0.14 [*]	7.08±0.11 [#]	7.01±0.1 [#]	7.16±0.12 [#]	6.85±0.1 [#]	7.02±0.13 [#]
Sodium (mmol/l)	138.3±2.1	153.52±3.2 [*]	143.9±2.4 [#]	144.03±2.3 [#]	139.36±2.2 [#]	134.97±2 [#]	146.41±2.4 [#]

Values are means ± S.E of 8-10 animals. As compared with control (*), nephrotoxic (#) groups, and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc test, P<0.05.

Table 2: Ameliorative effect of whey protein, *Nigella sativa* oil and their combination on body and kidney weights in potassium dichromate intoxicated rats.

Parameters	Control (vehicle)	Nephrotoxic (Pot.Di.Chr.)	Nephrotoxic (W prot. Low dose)	Nephrotoxic (W prot. Low dose + N.O.)	Nephrotoxic (W prot.High dose)	Nephrotoxic (W prot. high dose + N.O.)	Nephrotoxic (N.O.)
Body Weight (g)	140.28±1.25	136.85±1.5 [*]	167.57±2.6 [#]	168.28±2.9 [#]	177.28±3.1 [#]	176±3 [#]	179.42±2.8 [#]
Kidney Weight (g)	0.56±0.02	1.14±0.09 [*]	0.84±0.07 [#]	0.78±0.06 [#]	0.73±0.06 [#]	0.56±0.05 [#]	0.78±0.05 [#]

Values are means ± S.E of 8-10 animals. As compared with control (*), nephrotoxic (#) groups, and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc test, P<0.05.

Table 3: Protective effect of whey protein, *Nigella sativa* oil and their combination on renal tissue oxidative stress [catalase enzyme (CAT), malondialdehyde (MDA), glutathione (GSH) and super oxide dismutase enzyme (SOD)] levels in potassium dichromate intoxicated rats.

Parameters	Control (vehicle)	Nephrotoxic (Pot.Di.Chr.)	Nephrotoxic (W prot. Low dose)	Nephrotoxic (W prot. Low dose + N.O.)	Nephrotoxic (W prot.High dose)	Nephrotoxic (W prot. high dose + N.O.)	Nephrotoxic (N.O.)
CAT (U/g. tissue)	479.64±9.6	312.53±3.5 [*]	360.55±3.7 [#]	409.11±4.1 [#]	414.64±4.2 [#]	533.15±4.5 [#]	344.86±3.2 [#]
MDA (nmol/mg)	5.71±0.06	15.61±0.2 [*]	10.82±0.1 [#]	9.52±0.09 [#]	8.09±0.08 [#]	6.43±0.07 [#]	9.54±0.1 [#]
GSH (mg/g.tissue)	8.55±0.04	4.58±0.04 [*]	5.34±0.05 [#]	5.93±0.06 [#]	6.13±0.06 [#]	7.6±0.07 [#]	6.51±0.06 [#]
SOD (U/g. tissue)	244.16±3.6	180.87±2.4 [*]	198.38±2.6 [#]	208.56±2.9 [#]	205.78±2.8 [#]	225.09±2.7 [#]	202.22±3 [#]

Values are means ± S.E of 8-10 animals. As compared with control (*), nephrotoxic (#) groups, and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc test, P<0.05.

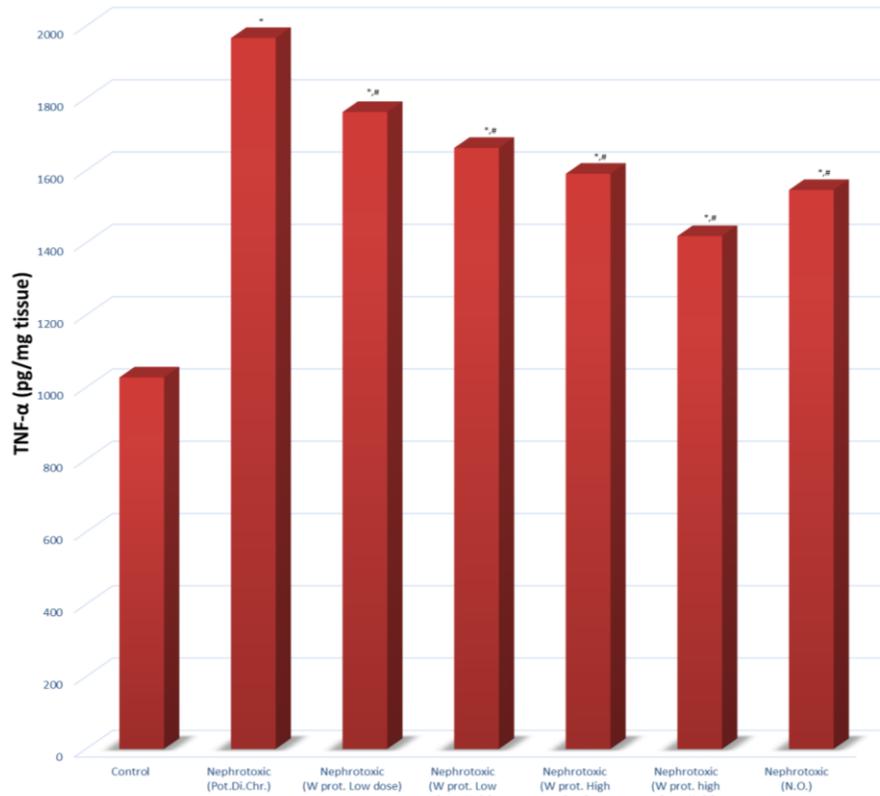


Fig. 1: Protective effect of whey protein, *Nigella sativa* oil and their combination on renal tissue TNF- α level in potassium dichromate intoxicated rats. Values are means \pm S.E of 8-10 animals. As compared with control (*), nephrotoxic (#) groups, and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc test, $P < 0.05$.

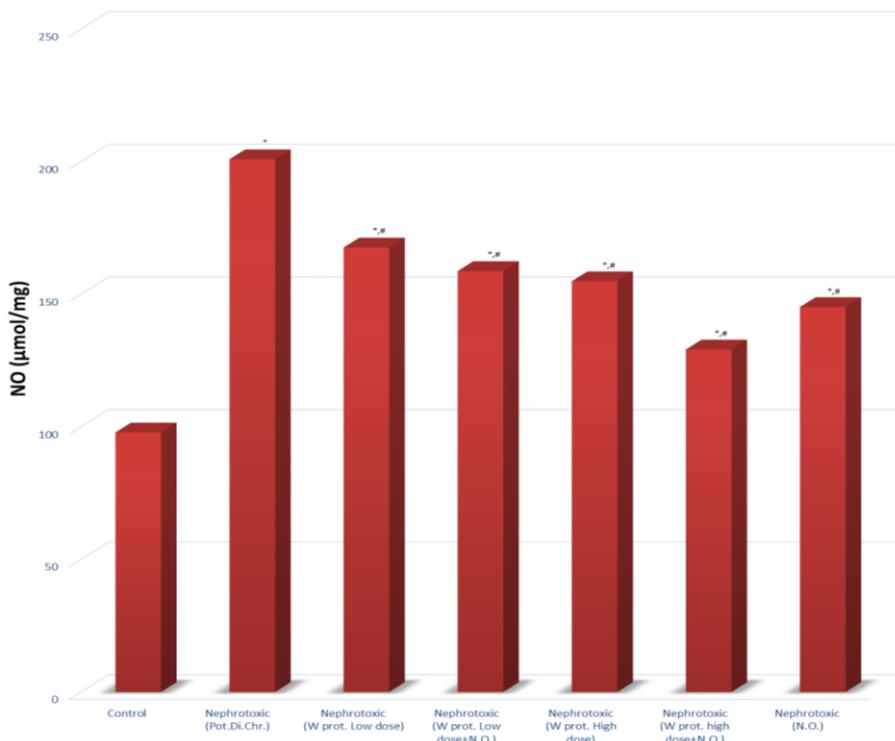


Fig. 2: Protective effect of whey protein, *Nigella sativa* oil and their combination on renal tissue NO level in potassium dichromate intoxicated rats. Values are means \pm S.E of 8-10 animals. As compared with control (*), nephrotoxic (#) groups, and the variances between groups were tested for significance using analysis of variance (ANOVA), followed by Tukey-Kramer post-hoc test, $P < 0.05$.

Histopathological examination

Histologically, the normal renal parenchyma consists of four parts: glomeruli, tubules (proximal tubules & distal tubules), interstitium and blood vessels shows in (Figure 3A).

Microscopic examination for kidney tissue from rats treated with potassium dichromate showing eosinophilic casts within the lumen of the tubules and hyaline droplet degeneration and necrosis in renal tubular epithelium. Signs of degeneration in tubular epithelial cells in the form of vacuolation, pyknosis and congested peritubular capillaries. Congested glomerular capillaries and atrophy of the glomerular tufts with mesangial necrosis. Massive inflammatory mononuclear cellular infiltration in interstitium (Figure 3B).

In the case of rats treated with potassium dichromate subjected to whey protein (low dose) showing thickening in the tubular epithelial cells and narrowing in the lumen of tubules. Signs of degeneration in tubular epithelial cells in the form of pyknosis and vacuolar degeneration in most of tubules. Congested of glomerular capillaries, and congestion of interstitial tissue. Some glomerular degeneration was observed (Figure 3C,D).

Concerning the rats treated with potassium dichromate along with whey protein (high dose) showing some improvement in pathological changes in the form of no inflammatory cells no eosinophilic casts within the lumen of the tubules and no congested peritubular capillaries. Although vacuolation in some tubular epithelial cells and lobulation of glomeruli with wide

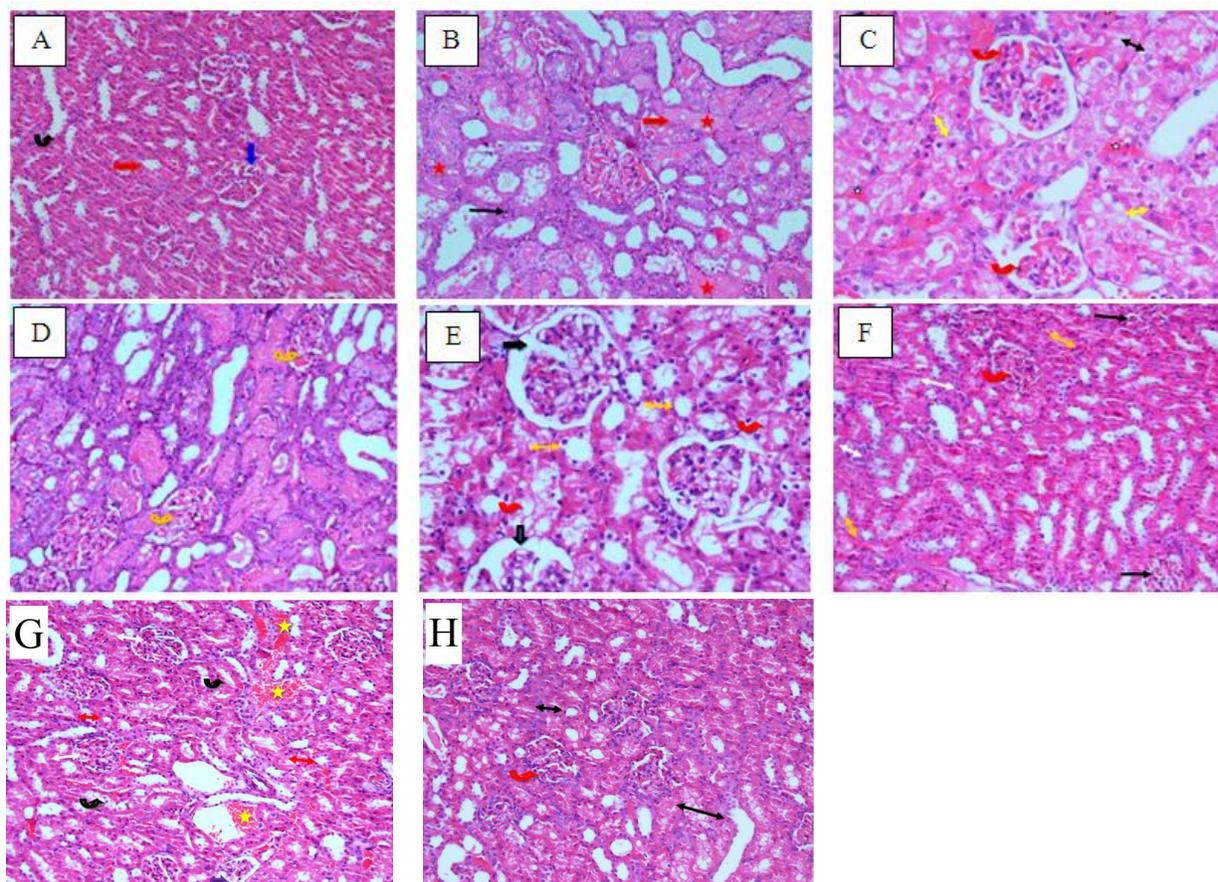


Fig. 3: Protective effect of whey protein, *Nigella sativa* oil and their combination on the histopathological state of kidney in potassium dichromate intoxicated rats. Fig. 3A: Section of kidney of control rat showing normal structure of renal glomeruli (blue arrow), Bowman capsule lined by squamous epithelium, distinct urinary space. The proximal tubules are lined with cuboidal epithelium with brush border and distal convoluted tubules with low cuboidal epithelium. **Fig. 3B:** Section of kidney of rat treated with potassium dichromate showing eosinophilic casts within the lumen of the tubules (star) Signs of degeneration in tubular epithelial cells in the form of vacuolation (red arrow), pyknosis (black arrow). **Fig. 3 C, D:** Section of kidney of rat treated with potassium dichromate along with whey protein at (low dose) showing thickening in the tubular epithelial cells and narrowing in the lumen of tubules. Signs of degeneration in tubular epithelial cells in the form of pyknosis (yellow arrow) and vacuolar degeneration (black arrow). Congested of glomerular capillaries (red curved arrow), and congestion of interstitial tissue (star) and congested glomerular capillaries and atrophy of the glomerular tufts with mesangial necrosis. **Fig. 3E:** Section of kidney of rat treated with potassium dichromate along with whey protein at (high dose) showing some improvement in pathological changes in the form of no inflammatory cells no eosinophilic casts within the lumen of the tubules and no congested peritubular capillaries but lobulation of glomeruli (black arrow) with wide urinary space were seen. The tubular epithelial cells with vacuolar degeneration (red arrow) and pyknosis could be observed (yellow arrow). **Fig. 3F:** Section of kidney of rat treated with potassium dichromate along with whey protein at (low level) and oil of *Nigella sativa* showing most of distal and proximal tubules appeared normal except interstitial tissue hemorrhage and (yellow arrow) inflammatory infiltrate (white arrow) shrinkage of some glomeruli (black arrow). **Fig. 3G:** Section of kidney of rat treated with potassium dichromate along with whey protein at (high dose) and oil of *Nigella sativa* exhibited most of distal and proximal convoluted tubules appeared normal. Hemorrhage in interstitial tissue and in interglomeruli was seen. **Fig. 3H:** Section of kidney of rat treated with potassium dichromate and oil of *Nigella sativa* revealed no histopathological changes, the kidney tubules (black arrow) and glomeruli (red arrow) appeared normal. (Hx&Ex200).

urinary space still present. Most of tubules appeared normal (Figure 3E).

Examination of tissue of kidney of rats treated with sodium dichromate subjected to whey protein at low level and oil of *Nigella sativa* showing most tubules appeared normal except interstitial tissue hemorrhage and shrinkage of some glomeruli and others with interglomerular hemorrhage were seen. Signs of degeneration in the form of pyknosis in some of tubular epithelial cells (Figure 3F).

Microscopic examination in kidney of rats treated with sodium dichromate along with whey protein at high dose and oil of *Nigella sativa* exhibited most of distal and proximal convoluted tubules appeared normal. Hemorrhage in interstitial tissue and in interglomeruli was seen (Figure 3G).

In the case of rats treated with potassium dichromate along with oil of *Nigella sativa* revealed no histopathological changes, the kidney tubules and glomeruli appeared normal (Figure 3H).

DISCUSSION

$K_2Cr_2O_7$ is a hexavalent form of Cr and it has been used in induction of renal oxidative stress (Elshazly *et al.*, 2015; Haney, 2015; Bucher, 2007). And it is reported that acute exposure induces anatomical lesions at the proximal tubular cells and lipid peroxidation in human kidney (Hose *et al.*, 2016). Chromium reduced intermediates were thought to react with hydrogen peroxide to form hydroxyl radicals (Bucher, 2007), with subsequent alterations in proteins, DNA, and phospho-lipids leading to disturbing cellular functions and its integrity (Bucher, 2007). So that, rats were intoxicated with $K_2Cr_2O_7$ with subsequent increase in the serum creatinine, urea, uric acid, proteins and sodium levels and severe alteration in the histopathological examination in comparison with normal control group, through the elevation of reactive oxygen species (ROS) that induces tissues damage such as liver, pancreas, cerebellum and kidney (Kim *et al.*, 2005; Fatima *et al.*, 2005). ROS generated by this process can bring on injury to cellular proteins, lipids, and DNA leading to oxidative stress (Li *et al.*, 2008; Mehany *et al.*, 2013).

By the same token, the incredible increase in MDA and NO and decrease of GSH, SOD and CAT is an excellent indicator for this oxidative stress through the activation of inducible nitric oxide synthase (iNOS), leading in over production of NO and generation of toxic peroxynitrite that reflected on losing body weight and kidney enlargement (Wang *et al.*, 2010; Molina-Jijon *et al.*, 2012). Another elucidation of nephrotoxicity induced by dichromate is the activation of inflammatory process as shown by elevated pro-inflammatory cytokine renal TNF- α level. This was proved before that hexavalent chromium could upshot ROS generation, induce the Akt, NF- κ B, and MAPK pathways beside elevation of cytokines, including TNF- α and IL-1 α levels (Zhao *et al.*, 2016; Takamura *et al.*, 2006). Whey protein treated diabetic group depicted a significant improvement in all plasma, tissue and histopathological parameters able to motivate the expression of the

enzymes linked to GSH synthesis, specifically gamma glutamyl cysteine synthetase (gamma-GCS), which is the key enzyme in GSH synthesis (Hesham *et al.*, 2012).

Whey protein, exhibited significant anti-inflammatory and antioxidant effects by increasing renal SOD, GSH, CAT and decreasing MDA, NO and TNF- α by its free radical scavenging properties which is reflected on kidney function (creatinine, urea, uric acid, sodium and proteins) (Mohebbati *et al.*, 2016). *Nigella sativa* oil showed a significant improvement in all altered parameters because it's have glutamic acid and methionine (Gholamnezhad *et al.*, 2016); this may explain its antioxidant effect. The beneficial effects of these oil and thymoquinone (one of its constituent) influence be contributed to their cyto-protective and antioxidant actions, by their effect on inflammation mediators.

CONCLUSION

In conclusion, our results elucidate that pretreatment with whey protein or *Nigella Sativa* Oil showed a potential antioxidant and anti-inflammatory properties in vivo by which they were able to reduce potassium dichromate induced nephrotoxicity. However, the exact and detailed mechanism of synergistic action of whey protein and *Nigella sativa* oil is not clear in earlier studies, so, we tried to spot light on its effect through different biomarkers to be further more investigated in the future.

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