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# Whey Protein and *Nigella sativa* Oil Mitigate Potassium Dichromate Induced Hepatic Injury, Oxidative Stress and Hematotoxicity in Rats

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## Abstract

The present study was carried out to evaluate the antioxidant property of whey protein and/or *Nigella sativa* Oil (NSO) against hepatotoxicity evoked by potassium dichromate($K_2Cr_2O_7$). Designed for this purpose, we detected the 8 weeks challenge result of whey protein (100 and 200 mg/kg, p.o) with/or without *Nigella sativa* oil (5ml/kg, p.o) in contradiction of poisoned albino rats with one dose of potassium dichromate (30mg/Kg, I.P) at the end of challenge period. Concerning plasma level, whey protein with/or without *Nigella sativa* oil were ameliorated the potassium dichromate liver damage concerns, so it exhibited a major progress in Aspartate Aminotransferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), and Gamma-Glutamyl Transferase (GGT). Moreover, whey protein or *Nigella sativa* oil reduce the deleterious effects of potassium dichromate on Triiodothyronine (T3), Thyroxine (T4), Thyroidstimulating Hormone (TSH), glucose and Complete Blood Count (CBC). In addition, they displayed an important improvement in hepatic antioxidant enzymes, Catalase (CAT) and Superoxide Dismutase (SOD) beside reduced Glutathione (GSH), and with a subsequent decrease in Malondialdehyde (MDA) or Nitric Oxide (NO) levels in comparison with the untreated $K_2Cr_2O_7$ group. Also, whey protein with/or without *Nigella sativa* oil improve the histopathological alterations produced by the potassium dichromate. These outcomes suggest that whey protein or *Nigella sativa* oil can be used as effective antioxidant aginst potassium dichromate intoxication as they modulate liver function and decrease oxidative stress.

**Keywords:** Hepatotoxicity, *Nigella sativa* Oil, Oxidative Stress, Potassium Dichromate, Whey Protein

## 1. Introduction

Human beings are subjected to a number of diverse chemicals that harm the liver. The liver has a noteable role in the metabolism of xenobiotics let this organ particularly liable to injury by chemicals to which we are exposed. The pathogenesis of most chemical-induced liver injuries is commenced by the metabolic transformation of chemicals into reactive intermediate species, such as free radicals, that can probably modify the structure and function of cellular macromolecules. Many reactive intermediate species can produce oxidative stress[1].

One of these hazardous hepatotoxic complexes is potassium dichromate, it is a powerful reacting mediator exhibiting a noticeable attraction, when converted to trivalent chromium ($Cr^{+3}$) through several cell reactions, to form a number of compounds with varied organic roots, together with nucleic acids[2]. Similarly, chromium triggered the production of Reactive Oxygen Species (ROS) which generate various poisonous properties, as well as DNA damage and phospholipid peroxidation that triggering hepatotoxicity[3].

Whey protein is a mixture derived from milk, containing lactoferrin, beta-lactoglobulin, alpha-

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lactalbumin, and glycomacropeptide, reveals a wide range of immune-enhancing mechanisms besides the facility to play as a powerful antioxidant and scavenging agent for free radicals[4]. Also, whey protein is containing a great proportion of cysteine and methionine, which improve immune system *via* intracellular conversion to glutathione. In addition, Lactoferrin, plays as an iron-chelating glycoprotein, acting a major character as an antioxidant[4].

*Nigella sativa* kernels include 36-38% stable oils, alkaloids, proteins and (0.5-2.6%) volatile oil. Investigational literatures have verified that *Nigella sativa* concentrate has a variety of medicinal properties used in treatment of hypertension, type 2 diabetes, immune-regulative and liver defense. *Nigella sativa* can afford important improvement of the hepatotoxic consequences of rats linked to its reactive oxygen species hunting and antioxidant effects[5].

Since whey protein and the *Nigella sativa* oil were appeared selected as essential supplements, the aim of our study is the evaluation of their proficiency to repairing liver injury and alleviating hematotoxicity accompanying potassium dichromate complications.

# 2. Material and Methods

## 2.1 Drugs and Chemicals

Whey protein (WP) was brought out from Davisco Food International Company, USA, whereas *Nigella sativa* oil (NSO) was purchased from Mepaco, Cairo, Egypt, and potassium dichromate ($K_2Cr_2O_7$)was bought from Sigma Aldrich, USA. Whey protein was administered orally, which suspended in distilled water.

## 2.2 Animals

Adult male Wistar albino rats weighing 130-140 g were obtained from the National Research Centre Laboratory (Dokki, Giza, Egypt) and were accommodated in standard polypropylene cages and kept under constant environmental conditions with equal light-dark cycles. Rats were adapted for 1 week and were served rat normal fat pellet diet and water ad libitum.

## 2.3 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. All surgery was performed under deep sodium pentobarbital anesthesia and all efforts were made to minimize suffering.

## 2.4 Induction of Hepatotoxicity using Potassium Dichromate and Experimental Design

70 rats were separated into 7 equal groups (Each group contained 10 rats) as follows:

Group 1: Control group received the vehicle.

Group 2: Rats injected with potassium dichromate only (30 mg/Kg IP, single injection)[6] and considered as a hepatotoxic group.

Group 3: Rats treated with 100mg/Kg whey protein (Low dose)[7] administered orally daily for 2 months, and then intoxicated with potassium dichromate (30 mg/Kg IP, single injection).

Group 4: Rats given both low dose of whey protein and *Nigella sativa* oil (5ml/Kg)[8], orally for two months, then intoxicated with potassium dichromate (30 mg/Kg IP, single injection).

Group 5: Rats treated with 200mg/Kg (high dose)[7] whey protein only daily for 2 months, then injected with potassium dichromate (30 mg/Kg IP, single injection).

Group 6: Rats administered a high dose of whey protein plus *Nigella sativa* oil, then intoxicated with potassium dichromate (30 mg/Kg IP, single injection).

Group 7: The seventh group administered orally *Nigella sativa* oil only for two months then intoxicated with potassium dichromate (30 mg/Kg IP, single injection).

Potassium dichromate and whey protein were dissolved in water. The samples were collected 24 hours post potassium dichromate injection.

## 2.5 Complete Blood Count (CBC)

CBC was done using Sysmex XT-1800i (Sysmex, Kobe, Japan), for determination of total leukocyte count (TLC), neutrophils (%), lymphocytes (%), eosinophils (%),

monocytes (%), Red Blood Cells (RBCs), Hemoglobin, Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC %), red blood cell distribution width (RDW%), Platelets (PLTs), and Mean Platelet Volume (MPV).

### 2.6 Multi-Component Spectrophotometric Method for the Simultaneous Determination of Four Hemoglobin Derivative Concentrations

Concentrations of Sulfhemoglobin (SHb), Methemoglobin (MetHb), Carboxyhemoglobin (HbCO) and Oxyhemoglobin ($HbO_2$) were measured by the multi-component method developed in our laboratory with some modifications. The hemolysate was prepared as described in this method. For absorbance measurements, about 30 µl of the purified hemolysate to 5 mL of temperature equilibrated (25°C) phosphate buffer ($Na_2HPO_4$ 27.50 mmol/L and $KH_2PO_4$ 13.16 mmol/L, pH 7.28) containing 0.4% Triton-X100. This technique has been applied in the simultaneous determination of SHb, MetHb, HbCO and the remaining functional Hb in the OxyHb form, with a conventional spectrophotometer. In this method, the measurements for the latter prepared extremely diluted Hb solutions were made, at four wavelengths (λ = 500, 569, 577 and 620 nm[9]).

### 2.7 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, 15 min) to separate the plasma. Colorimetric kits were bought from Salucea Company, Netherlands to determine serum AST, ALT, GGT, ALP, and glucose. ELISA kits were purchased for the assessment of serum T3, T4, and TSH from R&D Systems, USA.

### 2.8 Liver Tissue Extract

After blood collection, rats were decapitated under a deep sodium pentobarbital anesthesia. Rats' liver was separated out, washed, weighed and homogenized in Phosphate Buffer Solution [PBS] [10%]. Tissue homogenate was centrifuged at 1500×g at 4C° for 20 minutes and the supernatant was collected and stored at -80C° for the direct assessment of parameters.

### 2.9 Assessment of Hepatic Oxidative Stress Parameters

Hepatic malondialdehyde (MDA), Glutathione (GSH), Catalase (CAT), Superoxide Dismutase (SOD), and Nitric Oxide (NO) were determined using colorimetric kits obtained from Bio-diagnostic, Egypt.

### 2.10 Histopathological Examination of the Liver

Liver samples from all groups were fixed in 10% formol saline, embedded in paraffin, and dehydrated in ascending concentrations of ethyl alcohol (70-100%). Subsequently, 5u m tissue sections were cut, mounted on slides, stained with hematoxylin and eosin (H&E) for liver histopathology.

### 2.11 Statistical Analysis

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

## 3. Results

### 3.1 Liver Function Tests

In our work, next the poisoning of animals with potassium dichromate, animals displayed a condition of hepatotoxicity which was established by a substantial up-shot of plasma AST, ALT, GGT, and ALP levels in relation with normal group. In the meantime, hepatotoxic groups treated with either both doses of whey proteinand/or*Nigella sativa* oil demonstrated an obvious enhancement in these factors in comparison with an control hepatotoxic group (Table 1).

### 3.2 Complete Blood Count and the Percentage of Hemoglobin Derivatives Parameters

Also, next to intoxication of rats with potassium dichromate, animals showed a significant alteration in complete blood count [except monocytes, MCV, MCH, and MCHC (%)] and an increase in the percentage of hemoglobin

**Table 1.** Protective effect of whey protein, *Nigella sativa* oil and their combination on the activity of plasma hepatic enzymes (AST, ALT, GGT, and ALP) in different groups of potassium dichromate intoxicated rats

| Parameters | Control | $K_2Cr_2O_7$ | $K_2Cr_2O_7$+ 100 mg/Kg WP | $K_2Cr_2O_7$+ 100 mg/Kg WP+NSO | $K_2Cr_2O_7$+ 200 mg/Kg WP | $K_2Cr_2O_7$+ 200 mg/Kg WP+NSO | $K_2Cr_2O_7$+ 5ml/Kg NSO |
|---|---|---|---|---|---|---|---|
| AST (U/l) | 48.87±1.12 | 150.62±2.25$^{*}$ | 70±1.22$^{*\#}$ | 44.12±1.03$^{\#}$ | 47.5±0.95$^{\#}$ | 45±0.81$^{\#}$ | 65.87±1.18$^{*\#}$ |
| ALT (U/l) | 37±0.56 | 84±1.31$^{*}$ | 39.87±0.61$^{\#}$ | 41.37±0.68$^{\#}$ | 42.12±0.75$^{*\#}$ | 32.87±0.48$^{*\#}$ | 58.75±0.89$^{*\#}$ |
| GGT (U/l) | 3.24±0.1 | 6.47±0.21$^{*}$ | 5.26±0.18$^{*\#}$ | 4.96±0.16$^{*\#}$ | 4.3±0.15$^{\#}$ | 3.73±0.13$^{\#}$ | 4.85±0.19$^{*\#}$ |
| ALP (U/l) | 135.87±2.7 | 238.5±4.32$^{*}$ | 188.25±3.23$^{*\#}$ | 187.25±3.3$^{*\#}$ | 180±3.07$^{*\#}$ | 152.62±2.81$^{*\#}$ | 167.12±2.9$^{*\#}$ |

Values are means ± S.E of 6-10 animals. As compared with control (*), $K_2Cr_2O_7$ (#) groups, and the differences between groups were confirmed for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, at P<0.05.

derivatives [except $HbO_2$ (%)] compared with normal control group. Unexpectedly, all treated groups with whey protein or *Nigella sativa* oil + $K_2Cr_2O_7$ revealed a notable improvement in most of these aforementioned parameters in comparison with $K_2Cr_2O_7$ group. (Table 2 and 3).

## 3.3 Oxidative Stress Markers

Table 4 indicated important alterations in the antioxidant defense system of $K_2Cr_2O_7$ group as mirrored on the decrease of CAT, GSH and SOD with increase of MDA and NO levels compared with the normal control group.

**Table 2.** Effect of whey protein and/or*Nigella sativa* oil on the complete blood count (CBC) in different groups of potassium dichromate poisoned rats

| CBC | Control | $K_2Cr_2O_7$ | $K_2Cr_2O_7$+ 100 mg/Kg WP | $K_2Cr_2O_7$+ 100 mg/Kg WP+NSO | $K_2Cr_2O_7$+ 200mg/Kg WP | $K_2Cr_2O_7$+ 200mg/Kg WP+NSO | $K_2Cr_2O_7$+ 5ml/Kg NSO |
|---|---|---|---|---|---|---|---|
| TLC ($10^3$/mm$^3$) | 5.78±0.21 | 11.28±0.3$^{*}$ | 7.86±0.26$^{*\#}$ | 7.05±0.24$^{*\#}$ | 8.03±0.28$^{*\#}$ | 7.73±0.25$^{*\#}$ | 6.73±0.22$^{*\#}$ |
| Neutrophils (%) | 3.53±0.11 | 5.92±0.23$^{*}$ | 3.52±0.12$^{\#}$ | 3.64±0.13$^{\#}$ | 4.56±0.21$^{*\#}$ | 4.2±0.2$^{*\#}$ | 3.85±0.15$^{\#}$ |
| Lymphocytes (%) | 2.82±0.05 | 4.15±0.21$^{*}$ | 6.01±0.4$^{*\#}$ | 2.9±0.06$^{\#}$ | 2.85±0.04$^{\#}$ | 3.85±0.13$^{*\#}$ | 2.25±0.03$^{*\#}$ |
| Eosinophils (%) | 0.6±0.01 | 1±0.05$^{*}$ | 0.1±0.008$^{*\#}$ | 0.21±0.01$^{*\#}$ | 0.13±0.01$^{*\#}$ | 0.46±0.02$^{*\#}$ | 0.11±0.04$^{*\#}$ |
| Monocytes (%) | 0.46±0.02 | 0.5±0.03 | 0.36±0.01$^{*\#}$ | 0.41±0.02$^{\#}$ | 0.51±0.03 | 0.48±0.02 | 0.41±0.03$^{\#}$ |
| RBCs ($10^6$/mm$^3$) | 4.62±0.23 | 2.47±0.1$^{*}$ | 3.29±0.12$^{*\#}$ | 4.05±0.2$^{*\#}$ | 3.4±0.13$^{*\#}$ | 3.74±0.15$^{*\#}$ | 3.59±0.14$^{*\#}$ |
| Hemoglobin (g/dL) | 12.26±0.45 | 6.66±0.25$^{*}$ | 8.41±0.36$^{*\#}$ | 10.06±0.47$^{*\#}$ | 8.81±0.37$^{*\#}$ | 9.21±0.41$^{*\#}$ | 9±0.39$^{*\#}$ |
| MCV (fL) | 79.63±2.51 | 78.85±2.4 | 76.68±2.1 | 77.5±1.8 | 78.05±2 | 79.25±2.62 | 76.11±2.25 |
| MCH (Pg) | 27.43±1.15 | 25.65±0.9 | 25.48±1.05 | 25.15±1.2 | 25.88±1.6 | 26.48±1.36 | 25.1±1.25 |
| MCHC (%) | 33.3±1.25 | 32.06±1.06 | 33.3±1.32 | 33.06±1.14 | 33.15±1.1 | 33.43±0.97 | 32.98±1.13 |
| RDW (%) | 13.58±0.43 | 19.6±0.65$^{*}$ | 19.61±0.6$^{*}$ | 15.81±0.51$^{*\#}$ | 17.25±0.49$^{*\#}$ | 16.51±0.56$^{*\#}$ | 20.06±0.71$^{*}$ |
| PLTs ($10^3$/mm$^3$) | 300.5±6.22 | 644.83±10$^{*}$ | 302.16±5.6$^{\#}$ | 326±5.7$^{*\#}$ | 296.83±4.8$^{\#}$ | 359.33±6.1$^{*\#}$ | 330.66±6.3$^{*\#}$ |
| MPV (fL) | 8.71±0.34 | 6.23±0.18$^{*}$ | 7.31±0.21$^{*\#}$ | 7.43±0.23$^{*\#}$ | 7.6±0.26$^{*\#}$ | 7.4±0.24$^{*\#}$ | 8.25±0.31$^{\#}$ |

Values are means ± S.E of 6-10 animals. As compared with control (*), $K_2Cr_2O_7$ (#) groups, and the differences between groups were confirmed for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, at P<0.05.

**Table 3.** Effect of whey protein and/or*Nigella sativa* oil on the percentage of hemoglobinderivatives in different groups of potassium dichromate poisoned rats

| Hemoglobin derivatives | Control | $K_2Cr_2O_7$ | $K_2Cr_2O_7$+ 100 mg/Kg WP | $K_2Cr_2O_7$+ 100 mg/Kg WP +NSO | $K_2Cr_2O_7$+ 200mg/Kg WP | $K_2Cr_2O_7$+ 200mg/Kg WP+NSO | $K_2Cr_2O_7$+ 5ml/Kg NSO |
|---|---|---|---|---|---|---|---|
| SHb (%) | 0.23±0.01 | 0.31±0.01* | 0.12±0.005*# | 0.06±0.001*# | 0.15±0.006*# | 0.13±0.004*# | 0.17±0.007*# |
| Met-Hb (%) | 0.87±0.05 | 2.86±0.11* | 2.64±0.1*# | 1.62±0.08*# | 1.48±0.07*# | 2.08±0.09*# | 1.87±0.08*# |
| HbCo (%) | 0.88±0.04 | 2.71±0.12* | 1.62±0.09*# | 0.72±0.03# | 1.98±0.02*# | 1.45±0.07*# | 1.27±0.06*# |
| $HbO_2$ (%) | 97.8±3.25 | 93.4±3.12 | 112.26±4.1*# | 97.58±3.2 | 96.38±3.02 | 96.32±2.8 | 96.68±2.9 |

Values are means ± S.E of 6-10 animals. As compared with control (*), $K_2Cr_2O_7$(#) groups, and the differences between groups were confirmed for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, at P<0.05.

**Table 4.** Protective effect of whey protein, *Nigella sativa* oil and their combination on hepatic tissue oxidative stress [catalase enzyme (CAT), malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase enzyme (SOD)] levels in different groups of potassium dichromate intoxicated rats

| Parameters | Control | $K_2Cr_2O_7$ | $K_2Cr_2O_7$+ 100 mg/Kg WP | $K_2Cr_2O_7$+ 100 mg/Kg WP+NSO | $K_2Cr_2O_7$+ 200 mg/Kg WP | $K_2Cr_2O_7$+ 200 mg/Kg WP+NSO | $K_2Cr_2O_7$+ 5ml/Kg NSO |
|---|---|---|---|---|---|---|---|
| CAT (U/g. tissue) | 15.76±0.71 | 8.36±0.42* | 8.64±0.44* | 9.01±0.47* | 10.74±0.56*# | 12.22±0.63*# | 10.14±0.51*# |
| MDA (nmol/ mg) | 2.98±0.02 | 7.66±0.16* | 6.27±0.14*# | 5.61±0.12*# | 5.13±0.11*# | 5±0.09*# | 5.44±0.13*# |
| GSH (mg/g. tissue) | 7.72±0.21 | 4.21±0.12* | 4.91±0.15*# | 5.42±0.13*# | 5.33±0.14*# | 6.88±0.19*# | 6.09±0.17*# |
| SOD (U/g. tissue) | 239.87±4.1 | 168.75±3.6* | 186.88±3.7*# | 186.29±4.2*# | 194.66±3.8*# | 213.95±3.9*# | 189.55±3.5*# |
| NO (nmol/l) | 1.92±0.03 | 4.19±0.1* | 3.49±0.09*# | 3.6±0.08*# | 3.12±0.05*# | 2.84±0.06*# | 3.51±0.07*# |

Values are means ± S.E of 6-10 animals. As compared with control (*), $K_2Cr_2O_7$(#) groups, and the differences between groups were confirmed for significance using analysis of variance (ANOVA), followed by Tukey-Kramer posthoc test, at P<0.05.

Meanwhile, groups of $K_2Cr_2O_7$ treated with either whey protein and/or *Nigella sativa* oil demonstrated a superb clear improvement in these parameters in comparison with untreated hepatotoxic group except the hepatotoxic group treated with either small dose of whey protein or concomitant with *Nigella sativa* oil.

## 3.4 Hormonal Parameters

Potassium dichromate group manifested a significant remarked decline in serum T3, and T4 levels with a subsequent elevation in plasma glucose, and TSH levels in comparison with the normal group as presented in Table 5. The rats treated with whey protein or *Nigella sativa* oil + $K_2Cr_2O_7$ revealed a substantial improvement in these aforementioned markers level compared with the $K_2Cr_2O_7$ group, nevertheless the result of high dose of whey protein plus *Nigella sativa* oil was extra pronounced and obvious than other treated intoxicated rats.

## 3.5 Histopathological Examination

Microscopic picture of the liver of control rat showed the normal architecture of liver, comprising several hepatic lobules, each hepatic lobule formed of radially organized cords of liver cells from central vein to the margin of lobule separated by blood sinusoids (Figure 1a).

The section of liver treated with potassium dichromate at a dose of 30 mg/Kg revealed severe liver damage, including marked cytoplasmic vacuolar degeneration,

**Table 5.** Protective effect of whey protein, *Nigella sativa* oil and their combination on plasma glucose, T3, T4 and TSH levels of potassium dichromate intoxicated rats

| Parameters | Control | $K_2Cr_2O_7$ | $K_2Cr_2O_7$+ 100 mg/Kg WP | $K_2Cr_2O_7$+ 100 mg/Kg WP+NSO | $K_2Cr_2O_7$+ 200 mg/Kg WP | $K_2Cr_2O_7$+ 200 mg/Kg WP+NSO | $K_2Cr_2O_7$+ 5ml/Kg NSO |
|---|---|---|---|---|---|---|---|
| Glucose (mg/dl) | 85.44±2.56 | 133.43±6.87* | 105.00±3.98*# | 100.53±8.53# | 96.44±5.91# | 80.66±3.76# | 92.47±3.47# |
| T3 (ng/ml) | 3.8±0.07 | 1.32±0.06* | 1.66±0.02*# | 1.87±0.05*# | 2.24±0.08*# | 3.16±0.11*# | 3.00±0.04*# |
| T4 (ng/ml) | 4.50±0.14 | 2.34±0.07* | 2.58±0.03*# | 2.83±0.12*# | 4.00±0.11*# | 4.13±0.15*# | 3.62±0.07*# |
| TSH (ng/ml) | 5.00±0.16 | 17.03±0.73* | 12.65±0.47*# | 10.03±0.21*# | 9.24±0.18*# | 8.76±0.13*# | 11.09±0.32*# |

Values are means ± S.E of 6-10 animals. As compared with control (*), $K_2Cr_2O_7$ (#) groups, and the differences between groups were confirmed for significance using Analysis of Variance (ANOVA), followed by Tukey-Kramer posthoc test, at $P<0.05$.

shrunken nuclei, sings of nuclear degeneration in the form of necrosis, pyknosis, and karyolysis as well as distortion of hepatic cells (Figure 1b). Furthermore, a minute of vacuolar degeneration in the bile duct around the dilated portal tract (Figure 1c).

Animal treated with potassium dichromate plus a low dose of whey protein showed regular hepatic cords, the majority of hepatocytes around central vein appear healthy, however, slight dilation in central vein and blood sinusoids, sings of vacuolar degeneration as well as necrotic, pyknotic and karyolitic nuclei could be observed (Figure 1d).

On the other hand, the liver of the rats subjected to $K_2Cr_2O_7$ plus a high dose of whey protein showed few vacuolar and fatty degeneration around the portal area infiltrated by inflammatory cells, besides, sings of nuclear degeneration as necrosis, karyolysis, and karyorrhexis (Figure 1e).

The histological picture of the liver of rats treated with potassium dichromate and *Nigella sativa* oil exhibited a normal architectural pattern, most of the hepatocytes appeared healthy expect some of the hepatocytes around peripheral zone appeared vacuolated (Figure 1f).

## 4. Discussion

Potassium dichromate is a formula of chromium and it has stayed used in the induction of hepaticinjury[10–13]. And it is described that acute contact encourageshepatic structural alterationin the hepatocytes and phospholipid oxidation in the liver[13]. Chromium produced metabolites were supposed to be induced by $H_2O_2$ to form $OH^-$ radicals[14], with successive changes in amino acids, DNA, and lipids structure principal to changing cellular activities and itsstructure[14].

For that reason, rats were challenged with Potassium dichromate showed a subsequent increase in the serum AST, ALT, GGT, and ALP levels with highly changes in liver histopathological investigation compared with normal group, *via* increase of Reactive Oxygen Species (ROS) that triggers liver tissue injury[15]. ROS produced through this way can cause damage to tissue amino acids, phospholipids, and DNA principal to oxidative stress[16].

In the same way, the significant elevation in MDA and NO levels and the significant decline of GSH, SOD and CAT levels were are presentative markers for the elevated free radicals due to the stimulation of Inducible Nitric Oxide Synthase (iNOS), principal to excessive generation of NO and production of toxic peroxy-nitrite that indicated the overproduction of ROS[13,17].

The elevation of plasma activity of ALT, AST, and ALP is indicative of hepatocellular damage since the disruption of the plasma membrane leak intracellular enzymes into the bloodstream[18].

The increase in oxidative stress induced by potassium dichromate can explain hepatotoxicity and the alterations of CBC, the percentage of hemoglobin derivatives, glucose, and thyroid hormone levels. It was reported that chromium might elevate ROS production, trigger the Akt, NF-kB, and MAPK mechanisms alongside the increase

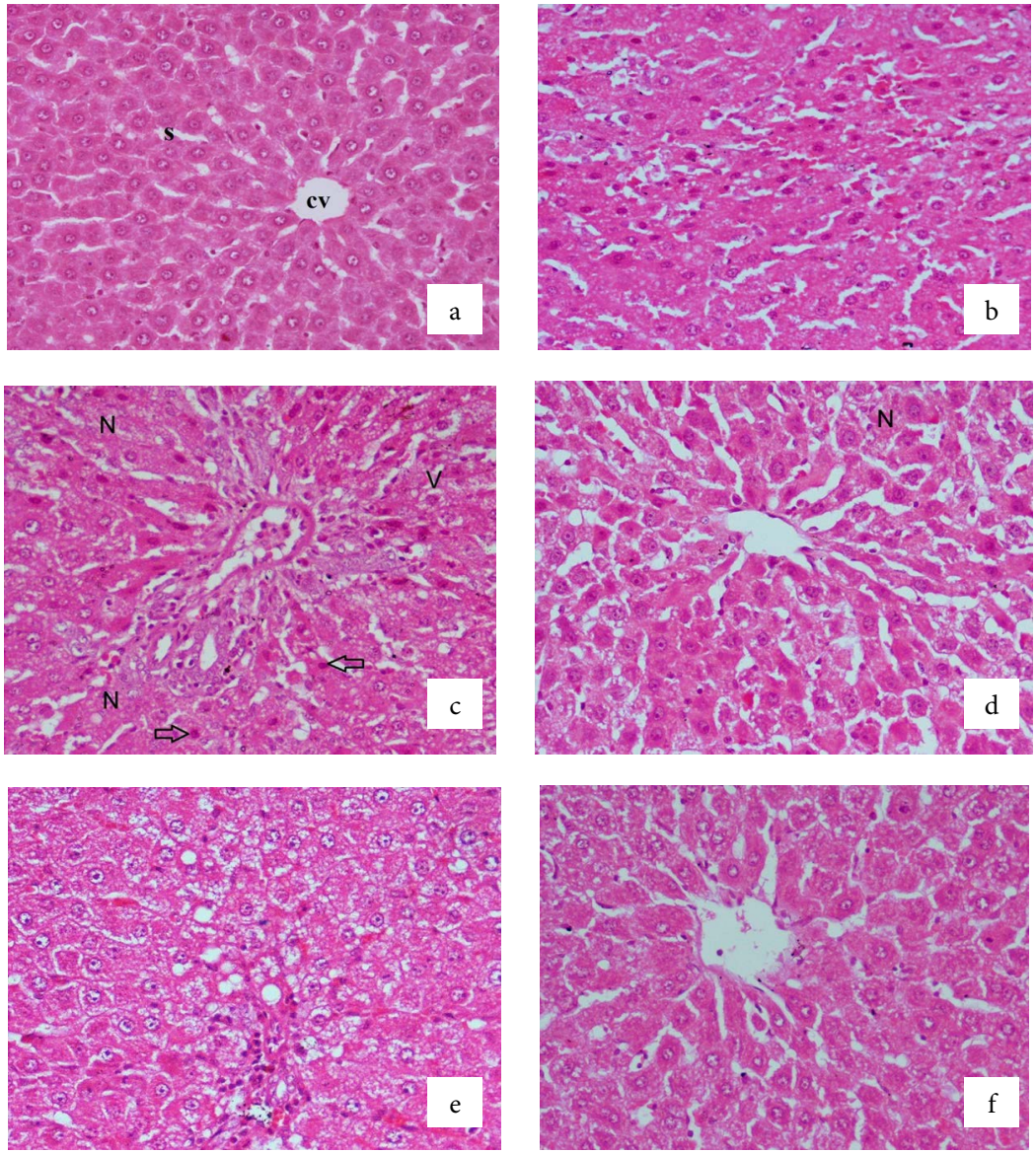


**Figure 1.** Effect of whey protein and/or *Nigella sativa* oil on the liver histopathological findings in different groups of potassium dichromate poisoned rats. **(a).** Section of the liver of control rat showing the normal structure of hepatic lobule, cords of cells radiated from acentral vein (cv), separated by blood sinusoid (s). **(b).** Section of liver treated with K.dichromat at dose 30 mg/Kg revealed marked damage in liver tissue, distortion of hepatic cells, shrunken nuclei, cytoplasmic vacuolar degeneration, sings of nuclear degeneration in the form of necrosis, pyknosis, and karyolysis. **(c).** Section of liver treated with K.dichromat at dose 30 mg/Kg showing cytoplasmic vacuolar degeneration (v), focal necrosis(N), nuclear degeneration in the form of pyknosis (arrow), karyolysis and increase in basophilia, slight dilatation of portal area and minute vacuolar degeneration in the bile duct around the dilated portal tract. **(d).** Section of liver treated with K.dichromat at dose 30 mg/Kg plus whey protein (Low dose) showing regular hepatic cords , the majority of hepatocytes around central vein appear healthy ,while, slight dilation in central vein and blood sinusoids, sings of vacuolar degeneration and necrotic (n), pyknotic as well as karyolitic nuclei could be observed. **(e).** Section of liver treated with K.dichromat at dose 30 mg/Kg plus whey protein (High dose) showing minute vacuolar degeneration, and fatty degeneration around the portal area infiltrated by inflammatory cells, besides, sings of nuclear degeneration as necrosis, karyolysis and karyorrhexis also were seen. **(f).** Section of the liver of rats treated with K. dichromat at dose 30 mg/Kg plus Nigella S. Oil exhibiting some improvement, most of the hepatocytes appeared healthy and the radial arrangement around the central vein wasrestored, although slight dilatation of central vein and blood sinusoid and minute vacuolation of peripheral hepatocytes still present. (Hx&Ex200).

of cytokines[19,20]. Our results showed that potassium dichromate produced a toxic effect on hematological parameters such as total erythrocyte count, total leucocyte count, and hemoglobin value. Potassium dichromate altered the erythropoiet in factors signifying anemia as evidenced by the decrease in the count of erythrocytes and hemoglobin concentration. The decrease in hemoglobin concentrations can be attributed to structural alteration of heme or to the resistance of the enzyme mechanism convoluted in the synthesis of hemoglobin, as proposed previously with other heavy metals[21].

It was reported that the ability of why protein to induce the expression of the enzymes associated with GSH synthesis might represent an important mechanism for the protective effect of whey protein[22]. Glutathione protects cells against exogenous and endogenous toxins, including reactive oxygen species and reactive nitrogen species[23,24].

Also, whey protein complex showed a substantial anti-inflammatory and antioxidant properties *via* elevating hepatic SOD, GSH, CAT and declining MDA, and NO through its ROS chelating effects which is evidenced from the improvement of liver biomarkers (AST, ALT, GGT, and ALP)[25], CBC, the percentage of hemoglobin derivatives, glucose, and thyroid hormone levels.

*Nigella sativa* oil revealed an important enhancement in entirely affected markers since it has antioxidant scavenging activity[26]. The advantageous effects of this oil influence are to be linked to their cytoprotective and anti-oxidant activities, by their influence on inflammatory markers. *Nigella sativa* oil exhibited a hepatoprotective effect against carbontetrachloride[27].

In our investigation, the rats given Nigella sativa oil and whey protein in combination indicated the best significant outcome compared to corresponding separared groups.

## 5. Conclusion

Our results reveal that protection with whey protein and/or *Nigella sativa* Oil exhibited possible anti-oxidant and anti-inflammatory effects in albino rats, which they were capable to decrease chromium triggered hepatotoxicity and hematotoxicity. Nevertheless, the particular and full mechanistic effect of whey protein and *Nigella sativa* oil is not perfect in former literatures, so, we focused to spotlight on their action *via* diverse investigations to be added further explored in the forthcoming studies.

## 6. Conflict of Interest

The authors have declared that no competing interests exist.

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## 8. Author Contributions

All authors of the current manuscript contributed equally to accomplish different parts of this work.

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