Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Issue **S-5 Year 2023** Page **480:495***

Wheat Germ Oil Restores Testicular Function Through Modulation of Oxidative Stress in Male Adult Rats Exposed to Chromium VI

Nema A. Mohammed[1*], Marium M. Hussein[2], Hayam F. Elshafey[1], Heba M. Abdou[1]

[1]Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt.
[2]Department of Zoology, Faculty of Science,Omar El-Mokhtar University, Libya.
**Corresponding author's E-mail: Nema A. Muhammed*

Article History

Received: 06 June 2023
Revised: 15 Sept 2023
Accepted: 14 Oct 2023

Abstract

This research was outlined to assess the protective and therapeutic role of wheat germ oil against chromium VI -induced oxidative stress and testicular dysfunction in male adult rats through evaluation of semen picture, measuring the sex hormone levels, oxidative stress markers, DNA fragmentation percentage and histopathological changes in the testes. Twenty-eight adult Wister male rats were assigned into four equal groups: Group 1; Control, group 2; Cr VI, group 3; Cr VI + WGO, and group 4; WGO. WGO showed a significant increment in the RBC, Hb, Ht, WBC and Plts. WGO restored the levels of testicular antioxidant enzymes, NO, MDA as well as GSH. Also, WGO in-combination with Cr VI showed a significant ($p<0.05$) increase in the levels of testosterone, LH, GnRH hormones and 17β-HSD enzyme, while, FSH was decreased. Data showed that treatment of male rats with WGO and Cr VI caused an increase in sperm count, motility, and decrease in sperm abnormality. Combination of WGO with Cr VI revealed a decrease in the levels of TL, TC, TG and LDL–C, while, HDL–C was increased. Rats administrated with WGO in-combination with Cr VI exhibited a slight improvement in testicular DNA integration compared to Cr VI-treated group. Further, co-administration of WGO + Cr VI revealed a slight improvement in the pathological alterations; the cellular layers of the seminiferous tubules more or less near to the normal structure. It was concluded that wheat germ oil can be an effective antioxidant in modulating Cr VI-induced male infertility, and may lead to improve the male reproductive performance.

Keywords: *Cr VI, WGO, testis, oxidative stress, testosterone, LH, FSH, GnRH, 17β-HSD.*

1. Introduction

Chromium (Cr) is a transition metal that exists in a variety of oxidation states. The toxic kinetics and toxicity of trivalent chromium-Cr (III) and hexavalent chromium-Cr (VI) compounds differ (Hodjat et al., 2017). Cr (VI) compounds have been considered as more relevant in toxicology than Cr (III) compounds since Cr (VI) is highly reactive in biological systems and far more poisonous than Cr (III). The study of Cr (III) compounds has been substantial (Fernandez et al., 2018). Cr (VI), one of the top ten environmental heavy metal contaminants (Jiang et al., 2018), is utilized in over 50 industries, including chrome plating, welding, painting, metal finishing, steel fabrication, alloys, cast iron and wood treatment, tannery leather processing, and food additives. The acceptable limit for CrVI in drinking water has been set at 2 g/l by the United States Environmental Protection Agency (USEPA) and the World Health Organization (WHO) (Efsa and Panel, 2014). Dermatitis, nasal perforation, and skin and lung cancer (Xu et al., 2015), cardiovascular disorders (Kumar et al., 2017), neurotoxicity, liver (Boşgelmez et al., 2017), spleen (Teklay, 2016), renal damage (Hao et al., 2017), and reproductive organs (Elshazly et al., 2015) are also well-known health concerns of Cr toxicity. For several years, the reproductive toxicity of Cr was downplayed, despite the findings of a few epidemiological research and numerous experimental experiments (Navin et al., 2017).

Several health risks may occur due to exposure to Cr (VI) including carcinogenic, cytotoxic, immunotoxic, neurotoxic, hepatotoxic, nephrotoxic, and genotoxic effects as well as general environmental toxicity (Marouani et al., 2017).

Different environmental elements, such as drugs, pollution, radiation, and toxicants, have a high level of sensitivity in the male reproductive system (Biswas et al., 2017). Because polyunsaturated fatty acids are abundant in testicular membranes, they are vulnerable to oxidative damage. Membrane permeability is affected by oxidative damage to this polyunsaturated fatty acid, causing congenital defects in babies and functional changes in adults (Anan et al., 2017).

There is a growing awareness of the importance of plant oils are becoming more widely recognized as a food source, and global demand for them has risen as a result (Sultan et al., 2020). Wheat germ oil (WGO) is a natural supplement derived from the germ of the wheat seed, which is the most important portion of the grain. Vitamin E, the most effective antioxidant, is found mostly in wheat germ oil (Soliman et al., 2020). Other antioxidants included in WGO include oleic acid, linoleic and -linolenic acid (ALA), tocopherol (vitamin E), which protect against oxidative stress and other beneficial components like glutathione, sterols, flavonoids, and octacosanolspolicosanols and sterols (Akool, 2015).

Wheat germ is a nutrient-dense food that comprises 10–15 percent lipids, 26–35 percent proteins, 17 percent carbohydrates, 1.5–4.5 percent fiber, and 4 percent minerals (Brandolini et al., 2012). The oil content of the germ is approximately 11%, with a significant number of beneficial substances (Dunford, 2012). Among vegetable oils, wheat germ oil has the highest tocopherol concentration. Furthermore, the proportion of polyunsaturated fatty acids, primarily linoleic and linolenic acids, is very high (almost 80% of the oil) (Zou et al., 2018). WGO possesses anti-inflammatory and antioxidant properties, making it useful in the prevention and treatment of disorders with oxidative damage caused by free radicals (Abdel Gawad, 2015). WGO serves as a reproductive agent, an additive in natural foods, and a health and cosmetic product, according to numerous experimental research (Anwar & Mohamed, 2015).

The present study was carried out to investigate the efficiency of WGO in reducing the testicular dysfunction and oxidative stress in adult male rats exposed to Cr VI and to assess its therapeutic potential for treatment of infertility.

2. Materials And Methods

Chemicals

Potassium dichromate with a molecular formula ($K_2CR_2O_7$) and molecular weight of 294.20 g/mol was obtained from El-Jamhoureia Chemical Company, Alexandria, Egypt (Oxford laboratory, Mumbai, India). Hexavalent chromium (Cr VI) was dissolved in distilled water. Wheat germ oil (WGO) was commercially available as soft gel capsules and was obtained from Cedco Co., Alexandria, Egypt. WGO was dissolved in corn oil just before the application to the rats.

Animals

Twenty-eight adult *Wister* male albino rats (aged 12 to 14 weeks old) were obtained from the Faculty of Agriculture, Alexandria University, Egypt their weight ranged from 180-200g. Rats were housed in stainless steel wire bottom cages. Animals were kept in standard housing facilities (24±1°C, 45±5% humidity, and 12 h light/dark cycle). They were fed standard laboratory pellets and provided water *ad-libitum*. Animal's procedures were consistent with the guidelines of Ethics by the Public Health Guide for the care and use of laboratory animals (National Research Council, 1996).

Experimental design:

Animals were randomly divided into 4 groups (n=7 animals) as follows:

Group I: Control group: Rats of this group orally received 0.3ml corn oil daily for 60 days.

Group II: Cr VI group: Rats of this group orally received 0.5 ml Cr VI dissolved in distilled

water at a dose of 0.2 mg/kg BW daily for 60 days (Gad et al., 1986).

Group III: WGO+Cr VI group: Rats of this group were pre-treated orally with WGO at a dose of 54 mg/kg BW (Reddy et al., 2000) for 10 consecutive days and in combination with Cr (VI) at a dose of 0.2 mg/kg BW/day for 60 days. The administration of WGO preceded Cr VI by 30 minutes.

Group IV: WGO group: Rats of this group orally received 0.3ml WGO at a dose of 54 mg/kg BW, daily for 60 days.

Blood collection

After 24 hours from the end of the experimental period (60 days), rats fasted for 12 hrs then were euthanized with ether and sacrificed by cervical dislocation. A part of the blood samples collected in tubes containing anticoagulant EDTA to determine hematological parameters. Another part of the collected blood was placed into sterile tubes and serum was obtained by centrifugation of samples at 10,000 ×g for 20 minutes (Hettich Zentrifugen, Universal 32 R, Germany) and stored at -80 ºC until its use for the various parameters.

Tissue Preparation

Part of the testis were immediately isolated, cleaned from adhering matters, washed in ice-cold saline (0.9% NaCl), dried and stored at -20 °C. Then, 0.5 g of testis tissue was minced and homogenized (10% w/v) separatly, in ice-cold 50 mM potassium phosphate buffer at pH 7.4 in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 ×g for 20 min (Hettich Zentrifugen, Universal 32 R, Germany) at 4 ºC. The supernatants were stored at -80 ºC to determine the stress markers and the activity of antioxidant enzymes. Part from testis of each group were sliced and immediately fixed in 10% formalin for histological examination.

Hematological parameters

EDTA was used as an anticoagulant and blood tested shortly after collection. Red blood corpuscles (RBCs), hemoglobin (Hb), hematocrit (Ht %) and platelets were determined. White blood cells (WBCs), and lymphocyte were counted by a cell counter (27390 Alfa Swelab, Sweden).

Biochemical measurement.

Nitric oxide in the testis and MDA were assayed according to the method of Montgomery *et al*. (1961) and Kei (1978), respectively. Reduced glutathione content was determined by the method of Jollow *et al*. (1974). Superoxide dismutase (SOD; EC 1.15.1.1), Catalase (CAT; EC 1.11.1.6) and Glutathione peroxidase (GPx; EC. 1.1.1.9) of testes were assayed by the method of Misra & Fridovich, (1972), Aebi, (1974) and Chiu *et al*. (1976), respectively. Total protein concentration in tissue was estimated by the method of Gornall *et al*. (1949). The activity of 17β- hydroxysteroid dehydrogenase enzyme activity (17 β-HSD; EC 1. 1.1. 51) was estimated by the method of Bogovich & Payne (1980).

Determination of lipid profile

Total lipid concentration was estimated according to the method of ZÖllner & Kirsch, (1962). The total cholesterol level in plasma was assayed colorimetrically by using commercial kits (Spinreact, S.A. /S.A. U Ctra. Santa Coloma, Spain) according to the method of Naito (1984). The triglycerides level in serum was assayed colorimetrically by using commercial kits according to the method of Fossati & Prencipe, (1982). LDL can be determined by the enzymatic method according to Weiland & Seidel, (1983). The High-density lipoprotein level in plasma was assayed colorimetrically by using commercial kits (Bio Systems S.A Costa Brava30, Barcelona, Spain) according to the methods of Burstein *et al*. (1980).

Determination of serum testosterone, GnRH, FSH and LH

Testosterone, FSH and LH were assayed by automated enzyme immunoassay system (ALA-360) called immulite/immulite 1000 system which based on the methods described by Abraham, (1977), Santner et al. (1981) and Beitens et al. (1976), respectively. These methods of assay were based on immunoassay reactions (Antigen antibody reactions). Also, GnRH assay employs the competitive inhibition enzyme immunoassay technique according to Abraham (1977) method.

Analysis of sperm quality

The testis and epididymis were sampled at the end of the experiment. Spermatozoa obtained from the cauda epididymis were subsequently diluted with physiological solution (20 µl) at 37 °C. The sample was located in the Makler chamber (Sefi-Medical Instruments, Germany). Analysis was realized using a CASA System–Supervision (Minitüb, Tiefenbach, Germany) with Olympus BX 51 (Olympus, Japan) microscope. The sperm count (million/ml), motile ratio (%) and abnormal sperm morphology (ASM, %) were evaluated in the experimental groups according to the method of Dunson et al. (1999).

Isolation and detecting of testis tissue DNA fragmentation.

The DNA was isolated from rat testis using Thermo Scientific Gene JET Genomic DNA Purification Kit. Samples are digested with Proteinase K in either the supplied digestion or lysis Solution. RNA is removed by treating the samples with RNase A. The lysate is then mixed with ethanol and loaded on the purification column where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer. Changes in DNA concentrations of testis tissue were quantified colorimetrically by using Thermo Scientific NanoDrop™ 1000 Spectrophotometer and the quality of DNA is detected by agarose gel electrophoresis. The separated DNA fragments are stained and thus visualized in the gel by fluorescent dye (ethidium bromide) which binds tightly to the DNA double helix by intercalating between the bases, and glows when illuminated with ultraviolet light. Photos indicating where the separated DNA fragments end up could be taken for later analysis, according to (Torres & Kuhn, 1997).

Histopathology

The excised testes were isolated and washed with normal saline followed by 50 ml (4%) paraformaldehyde in phosphate- buffered saline (PBS), postfixed in 10% formalin for 7 days, and then paraffin embedded. Paraffin blocks were sectioned into 4-5 µm thick sections. Serial sections of testis were stained with haematoxylin and eosin (H & E) according to Drury and Wallington (1980) to be examined at 100 and 400× magnifications using a standard light microscope for histopathological changes.

Statistical analysis

All data were expressed as mean±S.E. of number of experiments (n=7). The statistical significance level of treatment effects was evaluated by one way analysis of variance (ANOVA) using SPSS version 22 (SPSS, IBM, USA) and Post hoc comparisons between groups were obtained by Duncan's Multiple Range Test (DMRT). Values were considered statistically significant when $P<0.05$ (Duncan, 1957).

3. Results and Discussion

Effect of chromium (VI), wheat germ oil (WGO) and their combination on the hematological parameters

Data summarized in Table 1 showed a significant ($p<0.05$) decrease in red blood cells count (RBCs), hemoglobin content (Hb), hematocrit value (Ht), white blood cell count (WBCs) and platelets in Cr (VI) -treated group compared to the control group. While, pretreatment with WGO and in-combination with Cr (VI) revealed significant ($p<0.05$) increase in RBCs, Hb, Ht, WBCs and platelets compared to Cr (VI) –treated group. Treatment of WGO alone caused significant ($p<0.05$) increase in Hb and platelets and insignificant ($p>0.05$) increase in RBCs, Ht and WBCs compared to the control group.

Table 1: Effect of Cr VI, WGO and their combination on hematological parameters

Parameters	Experimental groups			
	Control	Cr (VI)	Cr (VI)+WGO	WGO
RBCs ($\times 10^6$ cell /µl)	4.50 ± 0.16^{a}	2.92 ± 0.23^{c}	3.71 ± 0.41^{b}	4.60 ± 0.04^{a}
Hb (g/dl)	12.00 ± 0.30^{b}	8.04 ± 0.55^{d}	10.62 ± 1.07^{c}	13.05 ± 0.23^{a}
Ht (%)	41.20 ± 3.07^{a}	20.40 ± 2.50^{c}	31.60 ± 3.80^{b}	41.40 ± 4.08^{a}

WBCs ($\times 10^3$ cell/ µl)	8.20±0.85[a]	5.95±0.05[c]	7.23±0.06[b]	9.04±0.04[a]
Plts ($\times 10^3$cell / µl)	234.32±2.99[b]	202.63±3.45[d]	227.00±4.05[c]	253.63±5.54[a]

Values are expressed as means± S.E, n=7 for each group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P< 0.05

Effect of chromium (VI), WGO and their combination on the levels of testicular stress markers and antioxidant enzyme activities.

Table 2 revealed changes in the levels of testicular nitric oxide (NO), malondialdehyde (MDA) and reduced glutathione (GSH). Animals treated with Cr (VI) showed significant (p<0.05) increase in the levels of testicular NO and MDA and significant (p<0.05) decrease in the levels of GSH, SOD, CAT, and GPx compared to the control ones. In contrast, pretreatment with WGO and in-combination with Cr (VI) showed significant (P<0.05) decrease in the levels of testicular NO and MDA, while, showed significant (P<0.05) increase in the levels of GSH, SOD, CAT, and GPx compared to Cr (VI) –treated group. Treatment of adult male rats with WGO alone showed insignificant (P>0.05) decrease in the levels of testicular NO and MDA as well as insignificant (P>0.05) increase in the level of GSH SOD, CAT, and GPx as compared to the control.

Table 2: Effect of chromium (VI), WGO and their combination on the levels of testicular stress markers and antioxidant enzyme activities.

Parameters	Experimental groups			
	Control	Cr (VI)	Cr (VI)+WGO	WGO
No (µmol/g tissue)	10.23±0.23[c]	65.32±0.75[a]	21.32±0.75[b]	8.63±0.30[c]
MDA (nmol/g tissue)	21.35±1.84[c]	57.6±2.44[a]	33.6±1.84[b]	18.32±0.64[c]
GSH (µmol/g tissue)	43.20±2.35[a]	12.14±0.50[c]	31.70±1.47[b]	45.20±2.56[a]
SOD (U/mg protein)	69.20±2.24[b]	21.56±1.23[d]	44.46±1.39[c]	72.50±1.77[a]
CAT (U/g tissue)	49.46±1.98[b]	23.96±1.38[d]	33.96±0.84[c]	54.50±2.48[a]
GPx (U/mg protein)	63.96±2.37[b]	25.71±1.49[d]	41.01±1.98[c]	68.62±3.07[a]
Total protein (mg/g tissue)	115.32±0.75[b]	81.00±2.26[d]	99.63±0.60[c]	127.00±3.60[a]

Values are expressed as means± S.E, n=7 for each group.

Mean values within a row not sharing a common super script letter (a, b and c) were significantly different P< 0.05.

Effect of chromium (VI), WGO and their combination on testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), gonadotropin releasing hormone (GnRH)and 17β–hydroxysteroid dehydrogenase enzyme (17β–HSD).

Table 3 showed that treatment with Cr (VI) caused a significant (p<0.05) decrease in testosterone, LH, GnRH, and 17β–HSD enzyme. While, significant (p<0.05) increase in the FSH level compared to the control group. Pretreatment with WGO and in-combination with Cr (VI) showed significant (p<0.05) increase in the levels of testosterone, LH, GnRH hormones and 17β–HSD, while, a significant (p<0.05) decrease in the level of FSH was observed as compared to Cr (VI)–treated group. Animals treated with WGO alone showed significant (p<0.05) increase in the levels of testosterone, LH, GnRH hormones and 17β–HSD enzyme as compared to the control group.

Table 3: Effect of chromium (VI), WGO and their combination on testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), gonadotropin releasing hormone (GnRH)and 17β–hydroxysteroid dehydrogenase enzyme (17β–HSD).

Parameters	Experimental groups			
	Control	Cr (VI)	Cr (VI)+WGO	WGO
Testosterone (ng/ml)	6.43±0.11^{b}	0.76±0.04^{d}	4.72±0.03^{c}	7.67± 0.05^{a}
LH (ng/ml)	1.02±0.05^{b}	0.28±0.10^{d}	0.56±0.05^{c}	1.52 ±0.11^{a}
FSH (ng/ml)	2.12±0.07^{c}	6.32±0.07^{a}	3.03±0.07^{b}	2.22±0.07^{c}
GnRH (pg/g tissue)	12.60±0.34^{b}	5.67±0.64^{d}	9.37±0.84^{c}	13.39±0.80^{a}
(17β–HSD) (unit/mg protein)	17.00±1.13^{b}	5.18±0.74^{d}	9.04±0.49^{c}	19.97±1.19^{a}

Values are expressed as means± S.E, n=7 for each group.

Mean values within a row not sharing a common superscript letter (a, b, c and d) were significantly different P< 0.05.

Effect of chromium (VI), WGO and their combination on sperm analysis.

Effect of Cr (VI), WGO and their combination on sperm count, motility and abnormality are presented in Table 4. Cr (VI) treatment caused a significant (p<0.05) decrease in sperm count, motility and a significant (p<0.05) increase in sperm abnormality compared to the control group. In contrary, administration of Cr (VI) with WGO showed significant (p<0.05) increase in sperm count, motility and significant (p<0.05) decrease in sperm abnormality compared to Cr (VI) –treated group. It was noticed that the treatment with WGO restored the alterations in sperm parameters near to normal. Data showed that treatment of male rats with WGO alone caused insignificant (p>0.05) increase in sperm count and a significant (p>0.05) increase in motility, while, caused significant (p>0.05) decrease in sperm abnormality compared to the control group.

Table 4: Effect of Cr (VI), WGO and their combination on sperm parameters.

Parameters	Experimental groups			
	Control	Cr (VI)	Cr (VI)+WGO	WGO
Count (10^{6}/ml)	92.98±1.44^{a}	14.80±3.71^{c}	48.40±3.48^{b}	92.80±0.79^{a}
Motility (%)	84.33±0.56^{b}	15.63±0.54^{d}	61.17±0.60^{c}	92.17±0.95^{a}
Abnormality (%)	13.32±1.32^{c}	44.70±0.42^{a}	22.80±2.19^{b}	11.00±0.64^{d}

Values are expressed as means± S.E, n=7 for each group.

Mean values within a row not sharing a common super script letter (a, b, c and d) were significantly different P< 0.05.

Effect of Cr (VI), WGO and their combination on the levels of total lipid(TL), total cholesterol (TC), triglycerides(TC), low density lipoprotein (LDL –C) and high density lipoprotein (HDL–C).

Table 5 showed that rats treated with Cr (VI) alone showed a significant (p<0.05) increase in the levels of TL, TC, TG and LDL–C and a significant (p<0.05) decrease in HDL–C compared to the control group. Pretreatment with WGO and in-compination with Cr (VI), revealed a significant (p<0.05) decrease in the levels of TL, TC, TG and LDL–C, while a significant (p<0.05) increase in HDL–C compared to Cr (VI) –treated group. Rats' treatment with WGO alone showed significant (p<0.05)

decrease in the level of TC, TG, LDL –C and significant ($p<0.05$) increase in HDL–C with insignificant ($p>0.05$) decrease in TL compared to the control group.

Table 5: Effect of Cr (VI), WGO and their combination on the levels of total lipid(TL), total cholesterol (TC), triglycerides(TC), low density lipoprotein (LDL –C) and high density lipoprotein (HDL–C).

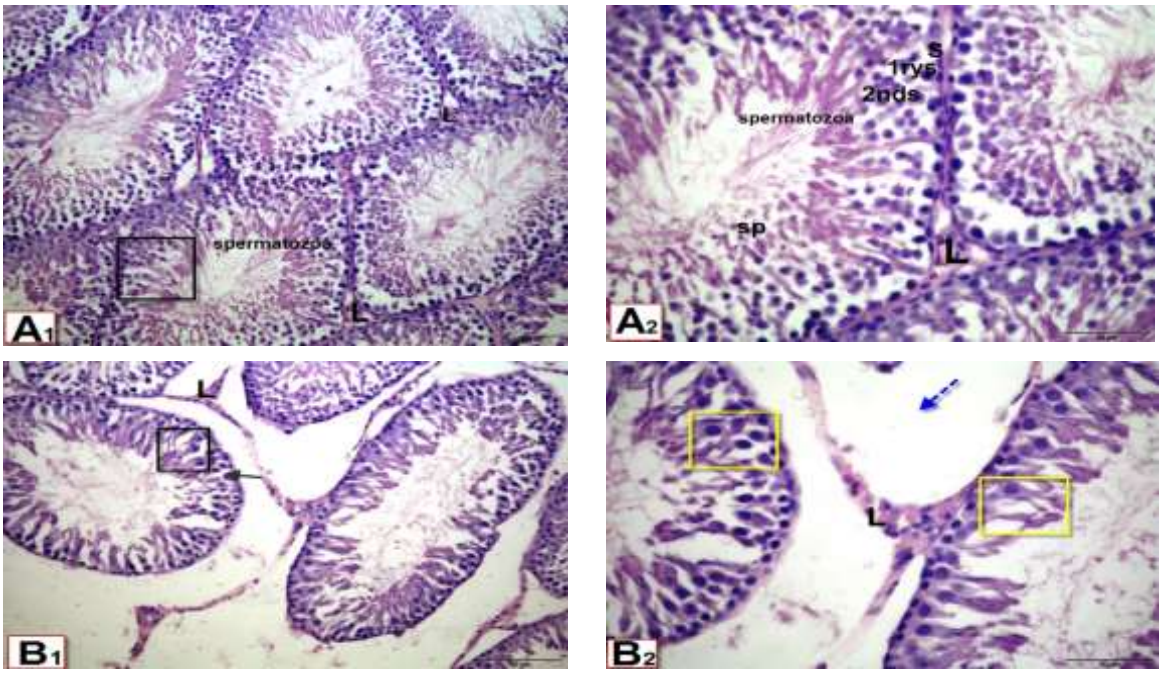
Parameters	Experimental groups			
	Control	Chromium(VI)	Chromium (VI) +WGO	(WGO)
TL (mg/dl)	315.00±0.93^{c}	684.32±3.89^{a}	405.00±3.41^{b}	309.32±2.49^{c}
TC (mg/dl)	49.03 ±0.31^{c}	146.74±1.67^{a}	65.05±2.31^{b}	32.45±0.65^{d}
TG (mg/dl)	72.63±2.84^{c}	142.32±0.75^{a}	97.00±0.93^{b}	66.32±2.39^{d}
LDL-C (mg/dl)	46.13±1.70^{c}	125.80±1.49^{a}	50.32±0.30^{b}	37.00±0.73^{d}
HDL-C (mg/dl)	44.17 ± 0.824^{b}	31.26 ± 0.683^{d}	38.43 ± 0.368^{c}	46.40 ± 0.441^{a}

Values are expressed as means± S.E, n=7 for each group.

Mean values within a row not sharing a common super script letter (a, b, c and d) were significantly different $P< 0.05$.

Effect of chromium (VI), wheat germ oil (WGO) and their combination on the histological pattern of the testis:

Photomicrographs of the testicular tissue from the control and WGO-treated group (Figures 1; A1 & A2 and D1 & D2) showed normal seminiferous tubules; complete stages of spermatogenesis (spermatogonia, primary and secondary spermatocytes, spermatidesand spermatozoa) as well as norml leydig cells. On the other hand, the testes of the Cr (VI)-treated group showed degeneration and disorganization of cellular layers of seminiferous tubules, few numbers of sperms and leydig cell atrophy (Figures B1 & B2) compared to the control group. While, administration of WGO+ Cr (VI) revealed a slightly improvement of these alteration (Figures C1 & C2).



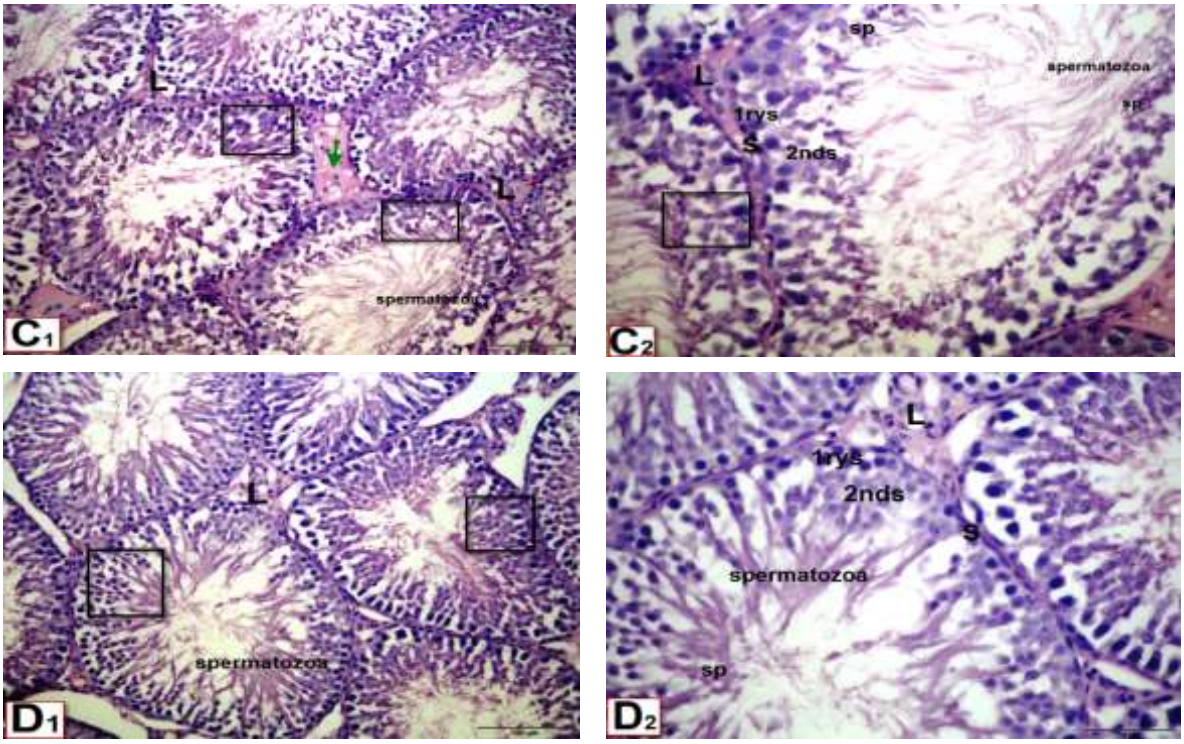


Figure 1: Photomicrographs of a T.S. rat testis, control group & WGO–treated group (A1&A2 and D1&D2) showing the normal morphological architecture of seminiferous tubules and Leydig cell (L), spermatogonia (S), primary spermatocyte (1^{ry} S), secondary spermatocyte (2^{ry} S), spermatids (sp) and lumen filled with spermatozoa. Cr (VI)–treated rats (B1&B2) showed marked degeneration and atrophy in most seminiferous tubules with the absence of spermatogenic stages and the presence of vacuoles (black arrow & yellow square). Lumen contains fewer spermatozoa. Atrophy in Leydig cell (L) with hemorrhage in interstitial tissues and enlargement space between seminiferous tubules (blue dotted arrow). Cr (VI)+WGO- treated rats (C1&C2) showed a marked improvement in the architecture of seminiferous tubules except for the presence of hemorrhage between seminiferous tubules (green arrow). Normal distribution of the spermatogenic cells (black square) and an increase in the number of spermatozoa (H&E, X 100 & X 400).

Changes in testicular DNA fragmentation of male rats by agarose gel electrophoresis.

Figure 2 showed a very low or undetectable DNA laddering (DNA fragmentation) of the control and WGO-treated rats (1^{st} and 4^{th} lanes, respectively). The DNA intact band appears to be condensed near the application point with no DNA smearing suggesting no DNA fragmentation. On the other hand, the Cr (VI)-treatment (2^{nd} lane) showed mild DNA fragmentations indicating testicular toxicity. Rats administrated WGO in combination with Cr (VI) slightly improves testicular DNA integration compared to Cr (VI)-treated group (3^{rd} lane).

G4 G4 G3 G3 G2 G2 G1 G1

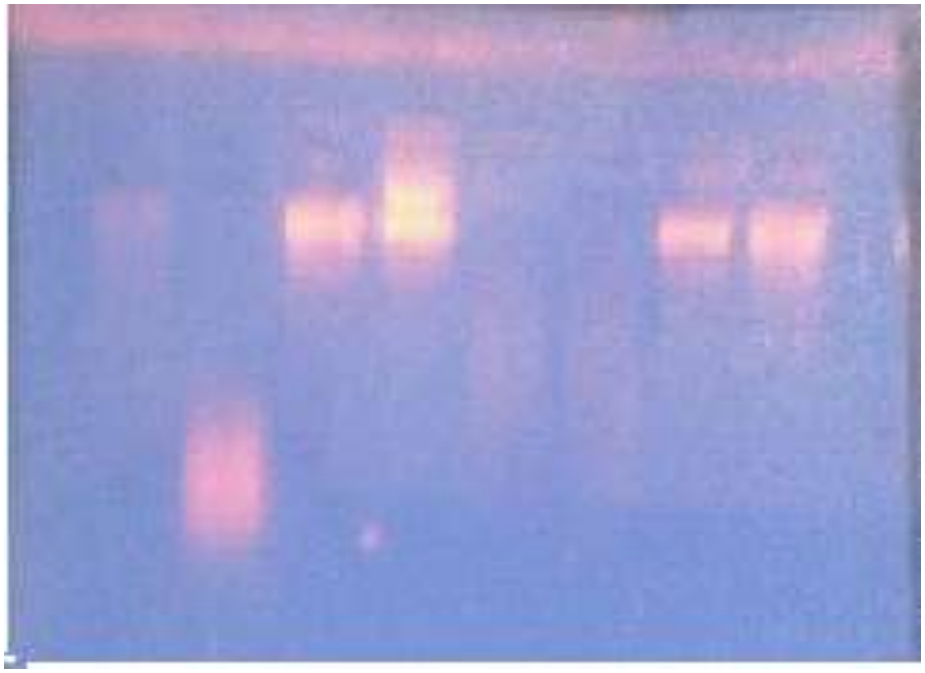


Fig 2: Electrogram demonstrating role of WGO against Cr (VI) induced genomic DNA fragmentation of rat testis. 1st lane, control; 2nd lane, Cr (VI) -treated rats; 3rd lane, rats treated with WGO plus Cr (VI); 4th lane, WGO-treated rats.

Cr (VI) has an anemic effect as documented by decreased red blood cells (RBC), hemoglobin (HB), hematocrit (Ht), white blood cells (WBC), and platelets, according to the findings of this study. These findings corroborated previous findings. This decline could be attributed to the fact that hexavalent chromium quickly enters erythrocytes and can accumulate in them, causing cell damage and erythropoiesis disorders (Jeevana, 2018; Sharmin, 2018) Furthermore, a drop in red Hb levels may be linked to a direct influence on iron metabolism, which may have a deleterious impact on the erythropoiesis process and lead to anemia and thrombocytopenia (Ognik, 2020). Furthermore, the drop in Hb level and RBC number seen in this study could be due to Cr (VI) binding to the beta-chain of hemoglobin in erythrocytes (Saidi, 2019), which explains the findings of Shahjahan (2020).

Because of the presence of Vitamin E in WGO, hematological indices improved to near-normal levels (Dahdouh et al., 2016). Vitamin E increased erythropoiesis and blood hemoglobin levels in these animals by preventing the oxidation of polyunsaturated fatty acids in RBC membranes (Jilani and Iqbal, 2018) Wheat germ oil also contains B complex vitamins (BR 6R, BR 12R, and folic acid), which are necessary for red blood cell formation and act as anti-inflammatory agents (AbdelFattah et al., 2016). WGO has been proven to be effective in avoiding oxidative stress against detrimental effects of hazardous compounds, and it was discovered that the group given wheat oil boosted the activation of erythropoietin in the adrenal gland (Abdou et al., 2017).

When the influence of chromium on white blood cell parameters was investigated, it was discovered that CrVI significantly reduced WBC count as well as lymphocyte and neutrophil. Leukopenia can occur as a result of diseases that develop in the bone marrow and disrupt hematopoiesis mechanisms (Sembratowicz and Ognik, 2018). Furthermore, excessive chromium Cr (VI) exposure activates radical oxidation processes and causes nutritional imbalances, which can lead to the development of immunological and metabolic abnormalities (Karaulov, 2019). Previous research has revealed that Cr (VI) causes immunosuppression, which increases disease sensitivity, inflammation, and cell damage (Yu et al., 2021).

Wheat germ oil may have mediated its effects by blocking free radical releases or scavenging these free radicals, resulting in a reduction in the damage generated in bone marrow cells. Wheat germ oil enhanced splenocyte growth (Vinod et al., 2012).

In the present data, Cr (VI) treatment revealed a dyslipidemic effect as manifested by high values of TC, TL, TG, LDL and low levels of HDL. Prior research has revealed that Cr (VI) can cause aberrant fatty acid metabolism in chicken livers, resulting in hepatotoxicity, which appears to be controlled by glucose and lipid metabolic pathways (Luo et al., 2018). Furthermore, epidemiological studies have shown that Cr (VI) exposure can cause considerable alterations in TG, FPG, LDL-C, Apo AI, and LP (a) levels in the blood (Feng et al., 2018). In rabbits, Cr (VI) has been shown to raise total lipids, cholesterol, and glucose levels (Soudani et al., 2013). Cr (VI) has also been shown in rats to raise FBG, TG, and LDL-C while lowering HDL-C levels (Banerjee et al., 2017).

Wheat germ oil is abundant with linolenic acid, which helps to promote cholesterol production and turnover by increasing cholesterol release into the bile and depletion of the intra-hepatic pool of cholesterol (Faghihimanl et al., 2016). Wheat germ oil may also help to minimize hepatic lipid buildup by promoting -oxidation and inhibiting fatty acid production (Brinton and Mason, 2017). WGO's essential fatty acids, particularly, have an antioxidant impact by blocking certain enzymes that facilitate the formation of free radicals, hence lowering the amount of free radicals produced. Also, WGO may have an anti-atherosclerotic impact by inhibiting oxidative stress-induced CD40 ligand upregulation (Alessandri et al., 2016). The presence of monounsaturated fatty acids, vitamin E, and phytosterol may cause WGO to reduce triacylglycerol. It could also be related to an increase in membrane permeability and fluidity, or to pancreatic lipase inhibition and reduced triacylglycerol lipolysis (Zakaria et al., 2017).

An imbalance between pro- and anti-oxidant species causes oxidative stress, which leads to molecular and cellular damage (Sushko et al., 2019). Studies have shown that Cr (VI)-induced toxicity is linked to oxidative stress (Zhang et al., 2017), which is mediated by reactive oxygen species (ROS). According to Zhang et al. (2019), Cr (VI)-induced mitochondrial dysfunction is accompanied by an increase in reactive oxygen species (ROS). Various ROS are formed after Cr (IV) enters the cell, according to studies. The cytotoxicity of chromium may be explained by their direct binding to cellular constituents, which is manifested by mitochondrial dysfunction, cell cycle interruption (Zhang et al., 2017), neoplastic transformation, or apoptosis induction.

DNA replication and transcription failure, dysregulation of DNA repair pathways, microsatellite instability, inflammatory responses, and impairment of genes involved in cell survival/death Fu et al., 2020) balance have all been linked to CrVI exposure (Fu et al., 2018). Hexavalent chromium, on the other hand, can cause DNA strand breakage, base oxidation, and the generation of Cr-DNA, DNA-Cr-DNA, or protein-Cr-DNA complexes, either directly or indirectly by the development of reactive oxygen species (ROS) (Fang et al., 2014).

In addition, Cr (VI) has been shown to increase SOD activity, raise MDA levels, and lower CAT activity in tissue (Xueting et al., 2018). The initial step in oxidative damage is the production of superoxide radicals. SOD is a dismutase enzyme that breaks down oxygen radicals. The increase in SOD activity seen in our study could represent an adaptive response to counteract the harmful effects of superoxide radicals produced by Cr (VI) (Balakrishnan et al., 2018). The decrease in CAT and GSH-Px activities could be due to Cr (VI)-induced reductions in NADPH and G6PD, whilst the increase in MDA levels could be due to lipid peroxidation (Ahmad et al., 2019).

Wheat germ oil has been used as an antioxidant source (Fine et al., 2016). The oral delivery of WGO resulted in a considerable drop in TBARs and a significant rise in blood serum antioxidant properties, according to the current data (CAT, SOD, and TAC). According to the current findings, the treatment WGO has a good effect on antioxidant status because they are very effective free radical scavengers (El-Sisy et al., 2018). WGO, on the other hand, reduces hepatic lipid peroxidation by boosting hepatic SOD activity (Niu et al., 2013). Wheat germ oil also has a beneficial effect on the antioxidant defense system, because to its high presence of natural antioxidants including phenolic compounds (Arslan et al., 2020), including, tocotrienols, alpha, beta, and gamma-tocopherols Carotenoids that are fat-soluble, such as lutein, zeaxanthin, and beta-carotene and fat-soluble carotenoids, such as lutein, zeaxanthin, and beta-carotene (Leenhardt et al., 2018). Furthermore, due to the individual and synergistic effects of fatty acids, antioxidants, vitamins, phytosterols, and phenolic compounds, WGO has outstanding antioxidant activities (Paranich et al., 2020). Another hypothesis is that WGO stimulates the tocopherol-

mediated redox system while inhibiting the formation of eicosanoids (prostaglandins) that increase lipid peroxidation (Chang et al., 2019).

For spermatogenesis and male reproductive function, the hypothalamic-pituitary-testicular axis must be intact (Tuttelmann et al., 2018). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are stimulated by gonadotropin-releasing hormone (GnRH) (LH). FSH and LH regulate testicular activity, which is required for the development of accessory sex organs. Chromium may affect spermatogenesis by altering the hormonal axis and increasing follicle-stimulating hormone (FSH) levels (Lei et al., 2001). Marouani et al. (2012) studied the effects of hexavalent chromium on male reproductive function in rats and discovered that it can raise FSH while, decreasing LH and testosterone levels in the blood. Furthermore, chromium exposure not only damages the Sertoli cell barrier, but it can also irreversibly damage the blood-testis barrier (Carette et al., 2013). As a result, chromium exposure may disrupt the hypothalamic-pituitary-testicular axis, affecting gonadotrophic hormone output. These alterations may have an impact on spermatogenesis, resulting in poor quality sperm (Kumar et al., 2017).

The effect of WGO on boosting serum testosterone, LH, and GnRH concentrations could be attributable to the fact that wheat germ oil includes vitamin E and phytoestrogen, which have the same activity in the blood as natural estrogen (Oluwatosin et al., 2018). Plant steroids were also reported to stimulate gonadotropin excretion in male and immature female rabbits, vitamin E in wheat-germ oil increased the development of gonads, testes, and ovaries in birds. Furthermore, the blood testosterone hormone was dramatically elevated in male animals due to the beneficial effect of vitamin E on testosterone release, which could be linked to better selenium and vitamin E utilization in the testes (Jerysz and Lukaszewiez, 2019). The considerable increase in the hormones LH and testosterone was confirmed by together with Muthu and Krishnamoorthy (2018). The activity of the LH and FSH hormones is determined by their levels as well as the number of specific receptors in the testicles. It has been established that the testis' Leydig cells are responsible for the manufacture and secretion of androgens, which are essential for male development and reproduction. In any event, FSH has an effect on sterol cells because it causes the synthesis of a testosterone-restricting protein in germ cells (Rengaraj and Hong, 2015). Furthermore, the inclusion of oleic acid, a monounsaturated fatty acid, in WGO reduces the testis' sensitivity to lipid peroxidation (Bourre et al., 2017).

Our idea could also be supported by histological findings in the testes and other reproductive organs, which revealed significant abnormal changes. Previous research has revealed that oxidative stress, lipid peroxidation, antioxidant defense system depletion, and increased production of pro-inflammatory mediators are implicated in the pathological changes of Cr-induced testicular toxicity (Fouad and Jresat, 2014). Cr also reduced the thickness of the epithelia of seminiferous tubules, which is thought to be due to degenerative processes and the deposition of sloughed cells in the tubule lumen (Jeber, 2013). Our findings are consistent with those of research on male reproductive organs in rats (Al-Mukhtar et al., 2016; Venter et al., 2017). According to Marouani et al. (2012) Cr (VI) subacute treatment promotes oxidative stress in the rat testis, which leads to apoptosis. In addition, Andleeb et al. (2018) concluded that Cr exposure causes testicular injury and potential reproductive system disturbances in mongooses because they observed disorganized seminiferous tubules, decreased numbers of Sertoli and Leydig cells, and decreased spermatozoa and seminiferous tubular counts (Sayantan and Shardendu, 2013). Similar studies found significant spermatid degradation after Cr administration, which could be due to damage to the testes blood barrier combined with Cr accumulation in the animal testis, or it could be due to the generation of reactive oxygen species (ROS) coupled with a disruption in the antioxidant defense system (Shahid et al., 2017).

The current findings revealed that giving WGO to male rats improved the quality and quantity of their sperm. WGO's beneficial effects may be attributed to its high vitamin E, -tocopherol, and other antioxidant concentrations. According to El-Banna et al. (2016), vitamin E has a protective effect against the toxicity of the testis caused by deltamethrin (DLM) in male rats, due to its powerful antioxidant action, Andleeb et al. (2018) concluded that vitamin E could preserve sperm DNA from oxidative stress and promote male fertility and spermatogenesis. In addition, Kumar et al. (2019) discovered that -tocopherol protects cells from apoptosis by reducing oxidative stress. WGO had a protective effect on body weight fluctuations and reproductive toxicity, according to Dina et al. (2020). Moreover, WGO was found to have a protective impact against body weight alterations and

reproductive toxicity, according to the study. Wheat germ oil can also help to repair testicular damage caused by heavy metal and heat (Khalaf et al., 2017). Wheat germ oil helps to maintain normal testicular function and protects against testicular damage.

4. Conclusion

The WGO has a greater restorative and protective effect on the testicular tissue toxicity of Cr VI in male rats.

Conflict of interest

The authors have declared no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Acknowledgment

The authors gratefully acknowledge Dr. Shaimaa Abdulla for molecular analysis, Medical Research Institute, Alexandria University

References:

Abdel Fattah, S.M., Fahim, T. & El-Fatih, N.M., 2011. Prophylactic role of combined treatment with wheat germ oil and ginseng against radiation injury in male rats. Egypt. J. Hosp. Med. 45,403–415.

Abdel-Gawad, S. K. (2015). Therapeutic and protective effect of wheat germ oil on l-arginine induced acute pancreatitis in adult albino rats. *Journal of Cell Science & Therapy*, (S8), 1.

Abraham, G.E. (Ed.). (1977). Handbook of radioimmunoassay. United States: Marcel Dekker, Inc.

Abdou, H.M., Mohamed, N.A., El Mekkawy, D.A., & EL-Hengary, SB., 2017. Vitamin E and/or wheat germ oil supplementation ameliorate oxidative stress induced by cadmium chloride in pregnant rats and their fetuses. Jordan J. Biol. Sci. 10(1), 39–48.

Aebi, H. (1974). Catalase (2nd ed.). *In* "Method of Enzymatic Analysis", Bergmeyer, H. U., Gawehn, K., Williamson, D. H. and Lund, P. (Eds.). Academic Press, Inc., New York and London. pp: 673–684. (Translated from the 3rd German ed.)

Ahmad, W.A., Venil, C.K., Nkhalambayausi Chirwa, E.M., Wang, Y.T., Sani, M., Samad, A. F. A., & Zakaria, Z. A., 2019. Bacterial reduction of Cr (VI): Operational challenges and feasibility. Curr. Pollut. Rep. 7(2),115–127.

Akool E., 2015. "Molecular mechanisms of the protective role of wheat germ oil against cyclosporin A-induced hepatotoxicity in rats". Pharm. Biol. 53(9), 1311–1317.

Alessandri, C., Pignattlli, P., Loffredo, L., Lenti, L., Del Ben, M. et al., 2016. Alpha-linolenic acid-rich wheat germ oil decreases oxidative stress and CD40 ligand in patients with mild hypercholesterolemia. Arterscl. Thromb. Vas. Biol. 26(11), 2577–2588. http://doi: 10.1161/01.ATV.0000242795.08322.fb.

Al-Mukhtar, N., Mohmmed, A., Mahdi, H., 2016. The effects of potassium dichromate on testes, accessory sex glands, liver and kidney in adult male rats treated with ascorbic acid. Aust. J. Basic. Appl. Sci. 10, 99–108. http://creative commons.org/licenses/by/4.0/

Andleeb, S., Mahmood, T., Khalid, A., Akrim, F., & Fatima, H., 2018. Hexavalent chromium induces testicular dysfunction in small Indian mongoose (*Herpestes javanicus*) inhabiting tanneries area of Kasur District, Pakistan. Ecotoxicol. Environ. Saf. 148, 1001–1009. http://doi: 10.1016/j.ecoenv.2017.11.075

Anan, H.H., Wahba, N.S., Abdallah, M.A., Mohamed, D.A., 2017. Antioxidant effect of selenium on hepatotoxicity induced by chlorpyrifos on male rats. Int. J. Pharm. Pharm. Sci. 4 (4), 603–609. http://doi.org/10.1007/s12011-013-9652-5

Anwar, M. M., & Mohamed, N. E. (2015). Amelioration of liver and kidney functions disorders induced by sodium nitrate in rats using wheat germ oil. J. Radiat. Res. Appl. Sci., 8(1), 77-83.

Arslan, D., Demir, M. K., Acar A., & Arslan, F. N., 2020. Investigation of wheat germ and oil characteristics with regard to different stabilization techniques. Food. Technol. Biotechnol. 58 (3), 348–-355. http://doi.org/10.17113/ftb.58.03.20.6638

Balakrishnan, R., Kumar, C. S.S., Rani, M. U., Srikanth, M. K., Boobalan, G., & Reddy, A. G., 2018. An evaluation of the protective role of α-tocopherol on free radical induced hepatotoxicity and nephrotoxicity due to chromium in rats. Indian J. Pharmacol. 45(5), 490.

Banerjee, N. S., Joshi, R., Mukherjee, P.K., Singh, D., & Baxi, A.V., 2017. Ramachandran Melatonin protects against chromium (VI) induced hepatic oxidative stress and toxicity: duration dependent study with realistic dosage. Inter. Toxicol. 10, 20–29. http://doi: 10.1515/intox-2017-0003

Beitens I.Z., O'loughlin K., Ostrea T. and Mcarthur J.W. (1976). Gonadotropin determination in the timed 3-hour mine cohectkms . during menstrual cycle The Journal of Clinical Endocrinology and Metabolism, 43: 46-55.

Biswas, A., D'souza, U. & Bhat, S., 2017. Dietary hypercholesterolemia induces oxidative stress challenging spermatogenesis in rat model: a link to possible infertility. Int. J. Pharm. Sci. Res. 8 (12), 5065–71.

Bogovich, K. and Payne, A.H. (1980): Purification of rat testicular microsomal 17-ketosteroid reductase. Evidence that 17-ketosteroid reductase and 17 beta-hydroxysteroid dehydrogenase are distinct enzymes. Journal of Biological Chemistry, 255(12):5552-5559·

Bourre, J., Dumont, O. & Durand, G., 2018. Dose-effect of dietary oleic acid: Oleic acid is conditionally essential for some organs. Reprod. Nutr. Dev. 44 (4),371–380. http://doi: 10.1051/rnd:2004042.

Brandolini, A., & Hidalgo, A. 2012. Wheat germ: Not only a by-product. Int. J. Food. Sci. Nutr. 63(1),71–4. http://doi.org/10.3109/09637486.2011.633898.

Brinton, E.A., & Mason, R.P., 2017. Prescription omega-3 fatty acid products containing highly purified eicosapentaenoic acid (EPA). Lipids Health Dis. 16,1–13.

Burstein, M., Scholnick, H. R. and Morfin, R. (1980). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *Scandinavian* Journal of Clinical and Laboratory Investigation, 40: 583–595.

Carette, D., Perrard, M.H., Prisant, N., Gilleron, J., Pointis, G., Segretain, D., & Durand, P., 2013. Hexavalent chromium at low concentration alters Sertoli cell barrier and connexin 43 gap junction but not claudin-11and n-cadherin in the rat seminiferous tubule culture model. Toxicol. Appl. Pharmacol.268(1), 27–36. http://doi :10.1016/j.taap.2013.01.016

Chiu, D. T. Y., Stults, F. H. and Tappel, A. L. (1976). Purification and properties of rat lung soluble glutathione peroxidase. Biochimica et Biophysica Acta, 445: 558–566.

Chang, C.L., Coudron, T.A., Goodman, C., Stanley, D.A.S., & Song, Q., 2019. Wheat germ oil in larval diet influences gene expression in adult oriental fruit fly. J. Insect Physiol. 56, 356–365. http://doi: 10.1016/j.jinsphys.2009.11.014

Dahdouh, F., Salah, A., Mohamed, R.D., & Zine, K., 2016. Effect of the joint supplementation of vitamin c and vitamin e on nickel heamatotoxicity and nephrotoxicity in male swiss albino mice. Int. J. Pharm. Pharm. Sci. 8(6),234–239.

Dina, A.S., Naglaa, E., Hanan, D.Y., Shereen, S.G., Batiha, G.E., & Nermeen, N.W., 2020. Pregabalin induced reproductive toxicity and body weight changes by affecting caspase3 and leptin expression: Protective role of wheat germ oil. Life. Sci. 260,118344. http://doi: 10.1016/j.lfs.2020.118344

Duncan, B. D. (1957). Multiple range test for correlated and heteroscedastic means. Biometrics, 13: 359–364.

Dunford, N. T., 2012. Traditional and emerging feedstocks for food and industrial bioproduct manufacturing. Food. Bioprod. Process. 1–35.

Dunson, D.B., Winberg, C.R., Perreault, S.D. et al. (*1999*) Summarizing the motion of self-propelled cells: applications to sperm motility. Biometrics, *55*, *537*–543.

Drury R. A. B. and Wallington E. A. (1980). Carleton's Histological Technique (5th ed.) Oxford University Press, Oxford New York Toronto, 188–189, 237–240, 290–291.

Efsa, P. P. R., 2014. Panel (EFSA Panel on Plant Protection Products and their Residues), Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA. J. 11(7), 3290.

EL Banna, H.A., Shalaby, M.A., Gehan, M.K., Galal, A.M., Marwa, S.K., & Azza, M.Z., 2016. Reproductive toxicity of deltamethrin in male rats and the protective role of vitamin E. Pharm. Chem. J. 3(3), 54–60.

Elshazly, M.O., Abd El-Rahman, S.S., Morgan, A.M., & Ali, M.E., 2015. The remedial efficacy of *Spirulina platensis* versus chromium-induced nephrotoxicity in male Sprague-Dawley rats. PLoS. One. 10,0126780. http://doi: 10.1371/journal.pone.0126780.

El-Sisy, G.A., Khalifa, W.H., El-Nattat, W.S., Abusinaa, G.E., & Maghraby, N.A., 2018. Effect of dietary supplementation of wheat germ on some reproductive performances and oxidative status of rabbit bucks under heat stress. Biosci. Res.15, 4267–4273.

Faghihimani, Z., Mirmiran, P., Sohrab, G., Iraj, B. & Faghihimani, E., 2016. Effects of pomegranate seed oil on metabolic state of patients with type 2 diabetes mellitus. Int. J. Prev. Med. 7,124. http://doi. org/10.4103/2008-7802.194883.

Fang, Z., Zhao, M., Zhen, H., Chen, L., Shi, P., & Huang, Z., 2014. Genotoxicity of tri- and hexavalent chromium compounds in vivo and their modes of action on DNA damage in vitro. PLoS. One. 9,103194. http://doi.org/10.1371/journal.pone.0103194.

Feng, F. H., Ha, G., Hu, Y., Wu, S., Yu, Z., Ji, W., Feng, T., Wang, G., Hao, P., Zhu, Y., Wang, S., Wan, H., Chen, P., Wang, Y., Cheng, Z., Liu, Y., & Liu, J., 2017. Selenium administration alleviates toxicity of chromium (VI) in the chicken brain. Biol. Trace. Elem. Res. 178,127–135. http://doi: 10.1007/s12011-016-0915-9.

Feng, H., Ha, F., Hu, G., Wu, Y., Yu, S., Ji, Z., & Jia, G., 2018. Concentration of chromium in whole blood and erythrocytes showed different relationships with serum apolipoprotein levels in Cr (VI) exposed subjects. J. Trace. Elem. Med. Biol. 50, 384–392. https://doi.org/10.1016/j.jtemb.2018.08.003.

Fernandez, P.M., Vinarta, S.C., Bernal, A.R., Cruz, E.L., & Figueroa, L.I.C., 2018. Bioremediation strategies for chromium removal: current research, scale-up approach and future perspectives. Chemosphere. 208,139–148.

Fine, F., Brochet, C., Gaud, M., Carre, P., Simon, N., Ramli, F., & Joffre, F., 2016. Micronutrients in vegetable oils: The impact of crushing and refining processes on vitamins and antioxidants in sunflower, rapeseed, and soybean oils. Eur. J. Lipid Sci. Technol. 118,680–697.

Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*, 28: 2077–2080.

Fouad, A. A., & Jresat, I., 2014. Thymoquinone therapy abrogates toxic effect of cadmium on rat testes. Andrologia. 47,417–26. https://doi.org/10.1111/and.12281

Fu, J., Liang, X., Chen, Y., Tang, L., Zhang, Q., & Dong, Q., 2018. Oxidative stress as a component of chromium-induced cytotoxicity in rat calvarial osteoblasts. Cell. Biol. Toxicol. 24,201–212. http://doi.org/10.1007/s10565-007-9029-7.

Gad, S.C., Powers, W.J., Dunn, B.J., Hoffman, G.M., Siino, K.M. and Walsh, R.D. (1986). Acute toxicity of four chromate salts. Serrone DM, Industrial Health Foundation Inc., 43–58.

Gornall, A. G., Bardawill, C. J. and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. The Journal of. biological. Chemistry, 177: 751-766.

Hao, P., Wan, H., Zhu, Y., Chen, P., Wang, Y., Cheng, Z., ... & Liu, J. (2017). Effect of various selenium doses on chromium (IV)-induced nephrotoxicity in a male chicken model. *Chemosphere*, *174*, 306-314.

Hodjat, M., Baeeri, M., Rezvanfar, M., Rahimifard, M., Gholami, M., & Abdollahi, M., 2017. On the mechanism of genotoxicity of ethephon on embryonic fibroblast cells. Toxicol. Mech. Methods. 27(3),173–80. http://doi:10.1080/15376516.2016.1273425

Jeber, Z. K., & Tawfeek, F. K., 2013. Effect of Turmeric Oil on Reproductive Efficiency of Adult Male Rats Exposed to Potassium Dichromate. IOSR. J. Environ. Sci. Toxicol. 3(4),52–58.

Jeevana, L.M., Srikanth, M.K., Gopala, R.A., & Anudeep, R.M., 2018. Haematological study in hexavalent chromium toxicity in female wistar rats and its progeny. Pharma. Innovation. 7(1),35–38.

Jerysz, A., & Lukaszewicz, E., 2013. Effect of dietary selenium and vitamin E on ganders' response to semen collection and ejaculate characteristics. Biol. Trace Elem. Res. 153(1-3), 196–204. http://doi: 10.1007/s12011-013-9652-5

Jiang, L.,Vincent,J.B.,&Bailey,M.M.,2018. Cr3O(O2CCH2CH3)6(H2O)3]NO3·H2O (Cr3) toxicity potential in bacterial and mammalian. cells. Biol. Trace. Elem. Res. 183(2),342–-350. http://doi: 10.1007/s12011-017-1132-x

Jilani, T., & Iqbal, M. P., 2018. Vitamin E deficiency in South Asian population and the therapeutic use of alpha-tocopherol (Vitamin E) for correction of anemia. Pak. J. Med. Sci. 34(6), 1571.

Karaulov, A. V., Renieri, E. A., Smolyagin, A. I., Mikhaylova, I. V., Stadnikov, A. A., Begun, D. N., et al., 2019. Long-term effects of chromium on morphological and immunological parameters of Wistar rats. Food Chem. Toxicol. 133,110748. http://doi: 10.1016/j.fct.2019.110748

Kei, S. (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica chimica acta, 90: 37-43.

Khalaf A. A., Ibrahim M. A., Tohamy A. F., et al., 2017. Protective effect of vitazinc on chlorsan induced oxidative stress, genotoxicity and histopathological changes in testicular tissues of male rats. Int. J. Pharmacol.13(1), 22–32. http://doi: 10.3923/ijp.2017.22.32

Kumar, A., Sharma, R., Rana, D., Sharma, N., 2019. Protective Effect of alpha-tocopherol in deltamethrin induced immunotoxicity. Endocr. Metab. Immune. Disord. Drug. Targets. 19(2),171–184. http://doi: 10.2174/1871530318666180801144822

Kumar, K.M, Aruldhas, M.M., Banu, S.L., Sadasivam, B., Vengatesh, G., Ganesh, K.M., Navaneethabalakrishnan, S., Navin, A.K., Michael, F.M., Venkatachalam, S., Stanley, J.A., Ramachandran, I., Banu, S.K., & Akbarsha, M.A., 2017. Male reproductive toxicity of CRVI: in-utero exposure to crviat the critical window of testis differentiation represses the expression of Sertoli cell tight junction proteins and hormone receptors in adult f1 progeny rats. Reprod. Toxicol. 69,84–98. http://doi: 10.1016/j.reprotox.2017.02.007

Leenhardt, F., Fardet, A., Lyan, B., Gueux, E., Rock, E., Mazur, A., Chanliaud, E., Demigné, C., Rémésy, C., 2018. Wheat germ supplementation of a low vitamin E diet in rats affords effective antioxidant protection in tissues. J. Am. Coll. Nutr. 27, 222–228. http://doi: 10.1080/07315724.2008.10719694

Lei, Z. M., Mishra, S., Zou, W., Xu, B., Foltz, M., Li, X., & Rao, C. V., 2001. Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. J. Mol. Endocrinol, 15(1), 184–200.

Luo, M., Huang, S., Zhang, J., Zhang, L., Mehmood, K., Jiang, J., ... & Zhou, D., 2019. Effect of selenium nanoparticles against abnormal fatty acid metabolism induced by hexavalent chromium in chicken's liver. Environ. Sci. Pollut. Res. 26, 21828–21834. https://doi.org/10.1007/s11356-019-05397-3

Marouani, N., Tebourbi, O., Hallègue, D., Mokni, M., Yacoubi, M.T., Sakly, M., Benkhalifa, M., & Rhouma, K.B., 2017., Mechanisms of chromium hexavalent-induced apoptosis in rat testes. Toxicol. Ind. Health.33, 97–106. http://doi: 10.1177/0748233715600333.

Marouani ,N., Tebourbi, O., Mahjoub, S., Yacoubi, M., Sakly, M., Benkhalifa, M., & Rhouma, K., 2012. Effects of hexavalent chromium on reproductive functions of male adult rats. Reprod. Biol. 12,119–133. http://doi: 10.1016/S1642- 431X(12)60081-3

Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of Biological Chemistry, 247: 3170–3175.

Montgomery, H.A.C. and Dymock, J.F. (1961). The determination of nitrite in water. *Analyst*, 86: 414-416.

Muthu, K., & Krishnamoorthy, P., 2018. Effect of vitamin C and vitamin E onmercuric chloride induced reproductivetoxicity in male rats. Bio. Chem. Pharm.1,7. http://doi: 10.4172/2167-0501.1000102

Naito, H. K. (1984). Cholesterol (1st ed.). In "Clinical Chemistry: Theory, Analysis and Co-relation", Kaplan, L. A. and Pesce, A. J. (Eds.). The C.V. Mosby, St. Louis, USA. pp: 437 and 1194–1206.

Navin, S. A.K., Navaneethabalakrishnan, S., Venkatachalam, M.A., Akbarsha, S. K., & Banu, M.M., 2017. Transient gestational exposure to hexavalent chromium (CrVI) adversely affects testicular differentiation: a study in rat model, J. Endocrinol. Reprod. 21, 93–108. https://doi.org/10.18311/jer/2017/23852

Niu, L.Y., Jiang, S. T., & Pan, L. J., 2013. Preparation and evaluation of antioxidant activities of peptides obtained from defatted wheat germ by fermentation. J. Food. Sci. Technol. 50, 53–61.

Ognik, K., Drażbo, A., Stępniowska, A., Kozłowski, K., Listos, P., & Jankowski, J., 2020. The effect of chromium nanoparticles and chromium picolinate in broiler chicken diet on the performance, redox status and tissue histology. Anim. Feed. Sci. Technol. 259,114326. http://doi; 10.1016/j.anifeedsci.2019.114326

Oluwatosin,V.I., Wuraola, A.E., Rasheed, A.A., Monsuru, O.A RSamson, A. Olusiji, F.S. & Oladele, S.G., 2018. Effects of Lepidiumsativum supplementation on growth and gonadotropins secretion in ovariectomized, estrogen-implanted rabbits. Asian. Pac. J. Reprod. 7(4), 155–160.

Paranich, V., Cherevko, O., Frolova, N., & Paranich, A., 2020. The effect of wheat germ oil on the antioxidant system of animals. Likars.' Ka. Sprav. 2, 40–44.

Reddy B, Hirose Y, Cohen L, et al. 2000. Preventive potential of wheat bran fractions against experimental colon carcinogenesis: implications for human colon cancer prevention. Cancer Res. **60:** 4792.

Rengaraj, D., & Hong, Y. H., 2015. Effects of dietary vitamin E on fertility functions in poultry species. Int. J. Mol. Sci. 16(5),9910–9921. http://doi:10.3390/ijms16059910

Saidi, M., Aouacheri, O., Saka, S., Tebboub, I., & Ailane, L., 2019Nephron-protective effects of curcuma on oxidative damage and oxidative stress in rat under sub-chronic poisoning of chromium. Int. J. Biosci. 15, 241–250. http://doi: 10.12692/ijb/15.1.241-250

Santner S., Santner R., Kulin H. and Demers L. (1981). A. model validation of radioimmuno-assay kit reagents: measurement of follitropin and lutrupin in blood and urine. Clincal Chemistry, 27: 1892- 1895.

Sayantan, D., 2013. Amendment in phosphorus levels moderate the chromium toxicity in Raphanus sativus L. as assayed by antioxidant enzymes activities. Ecotoxicol. Environ. Saf., 95, 161–170. http://doi: 10.1016/j.ecoenv.2013.05.037

Sembratowicz, I., & Ognik, K., Immunotropic activity of gold nanocolloid in chickens. J. Trace. Elem. Med. Biol. 47,98–103. http://doi: 10.1016/j.jtemb.2018.02.006

Shahid, M., Dumat, C., Khalid, S., Niazi, N.K., & Antunes, P.M.C., 2017. Cadmium Bioavailability, Uptake, Toxicity and Detoxification in Soil-Plant System. Rev. Environ. Contam. Toxicol. 241, 73–137. http://doi: 10.1007/398.2016.8

Sharmin, M.A.S., Salam, F., Haque, M.S., & Islam, M., 2018. Changes in hematological parameters and gill morphology in common carp exposed to sub-lethal concentrations of malathion, Asian. J. Med. Biol. Res. 2,370–378. http://doi: 10.3329/ajmbr.v2i3.30106

Shahjahan, M., Taslima, K., Rahman, M. S., Al-Emran, M., Alam, S. I., & Faggio, C., 2022. Effects of heavy metals on fish physiology–A review. Chemosphere.134519. http://doi: 10.1016/j.chemosphere.2022.134519.

Soliman, G.Z.A., 2020 Effect of wheat germ, wheat germ oil on lipid profile of hypercholesterolemic rats. J. Lipid Res. 1(1), 11–20.

Soudani, H. N., Bouaziz, M., Sefi, Y., Chtourou, T., & Boudawara, N., 2018. Toxic effects of chromium (VI) by maternal ingestion on liver function of female rats and their suckling pups. Environ. Toxicol. 28,11–20. http://doi: 10.1002/tox.20692

Sultan, F., Al-Farha A.A., & Shaaban. I., 2020. Separation and identification of some fatty acids and phenolic compounds from Portulaca oleracea L. and study their biological effect on two types of pathogenic bacteria. Asian J. Agric. Biol. 8,281–29.

Sushko, O.O., Iskra, R.J., Ponkalo, L.I., 2019. Influence of chromium citrate on oxidative stress in the tissues of muscle and kidney of rats with experimentally induced diabetes. Regul. Mech. Biosyst. 10(2),209–14. http://doi.org/10.15421/021931

Teklay, A. (2016). Physiological effect of chromium exposure: a review. Int. J. Food. Sci. Nutr. Diet. 7,1-11. http://dx.doi.org/10.19070/2326-3350-SI07001

Torres, R. M. and Kühn, R. (1997). Laboratory protocols for conditional gene targeting. Oxford University Press, Oxford.

Tuttelmann, F., Ruckert, C., & Ropke, A., 2018. Disorders of spermatogenesis:perspectives for novel genetic diagnostics after 20 years of un-changed routine. Med. Genet. 30(1),12–20. http://doi: 10.1007/s11825-018-0181-7

Venter, C., Oberholzer, H.M., Cummings, F.R., Bester, M.J., 2017. Effects of metals cadmium and chromium alone and in combination on the liver and kidney tissue of male Spraque-Dawley rats: An ultrastructural and electron-energy-loss spectroscopy investigation. Microsc. Res. Tech. 80,878–88. http://doi.org/10.1002/jemt.22877

Vinod, M., Jain, P., Verma, H., Kumar Gupta S., Sharma, V. &Thakur, R., 2012. An overview on natural treatment of systemic lupus erythematosus. Asian J. Pharm. 2,2231–4423.

Wieland, H., & Seidel, D. (1983). A simple specific method for precipitation of low density lipoproteins. Journal of Lipid Research, 24: 904-909.

Xu, X., Yekeen, T.A., Liu, J., Zhuang, B., Li, W., & Huo, X., 2015. Chromium exposure among children from an electronic waste recycling town of China. Environ. Sci. Pollut. Res. 22,1778–1785. http://doi: 10.1007/s11356-013-2345-y.

Xueting, L., Rehman, M. U., Mehmood, K., Huang, S., Tian, X., Wu, X., & Zhou, D. 2018. Ameliorative effects of nano-elemental selenium against hexavalent chromium-induced apoptosis in broiler liver. Environ. Sci. Pollut. Res. 25(16), 15609–15615. http://doi: 10.1007/s11356-018-1758

Yu, Z., Xu, S. F., Zhao, J. L., Zhao, L., Zhang, A. Z., & Li, M. Y., 2021. Toxic effects of hexavalent chromium (Cr) on bioaccumulation, apoptosis, oxidative damage and inflammatory response in *Channa asiatica*. Environ. Toxicol. Pharmacol. 87, 103725.

Zakaria, H., Mostafa, T.M., El-Azab, G.A., Abd El Wahab, A.M., Elshahawy, H., & Sayed-Ahmed, N.A.H., 2017. The impact of fish oil and wheat germ oil combination on mineral-bone and inflammatory markers in maintenance hemodialysis patients: a randomized, double-blind, placebo-controlled clinical trial. Int. Urol. Nephrol. 49, 1851–1858

Zhang, F. Y., Xiao, X., Liu, K., Liu, X., & Zhou, C., 2017. Cr(VI) induces cytotoxicity *in vitro* through activation of ROS-mediated endoplasmic reticulum stress and mitochondrial dysfunction via the PI3K/Akt signaling pathway. Toxicol. 41,232–244. http://doi: 10.1016/j.tiv.2017.03.003.

Zhang, Y. Y., Ma, N., Liang, Y., Liang, C., Lu, F., 2019. Blockage of ROS-ERK-DLP1 signaling and mitochondrial fission alleviates Cr(VI)-induced mitochondrial dysfunction in L02 hepatocytes Ecotoxicol. Environ. Saf. 186,109749. http://doi: 10.1016/j.ecoenv.2019.109749

Zöllner, N. and Kirsch, K. (1962). Colorimetric method for determination of total lipids. Zeitschrift Fur Die Gesamte Experimentelle Medizin, 135: 545-561.

Zou, Y., Gao, Y., He, H, Yang, T., 2018. Effect of roasting on physico-chemical properties, antioxidant capacity, andoxidative stability of wheat germ oil. LWT – Food. Sci. Technol. 90,246–53. https://doi.org/10.1016/j.lwt.2017.12.038