DOI: 10.56511/JIPBS.2019.v6.i04.pp23-32

Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Quinoa (*Chenopodium quinoa*) extract ameliorates hemato-cardio injuries induced by cyclophosphamide® in rats

Khaled G. Abdel-Wahhab[1*], Fathia A. Mannaa[1], Mahmoud Ashry[2], Mohamed A. Mustafa[3], Heba Fawzi[4], Laila K Hassan[5]

[1]*Medical physiology department, National Research Centre, Giza, Egypt.*
[2]*Zoology department, Faculty of Science, Al-Azhar University, Asyut, Egypt.*
[3]*Basic Centre of Science, Misr University for Science and Technology, Giza, Egypt.*
[4]*Zoology department, Faculty of Science, Ain Shams University, Cairo, Egypt.*
[5]*Dairy department, National Research Centre, Giza, Egypt.*

Key words: Cardiotoxicity, hematopoietic, Cyclophosphamide, Quinoa, Rat.

***Corresponding Author: Khaled G. Abdel-Wahhab,** Medical physiology department, National Research Centre, Giza, Egypt.

Vol. 6 (4), 23-32, Oct-Dec, 2019.

Abstract

Due to the cytotoxic properties of cyclophosphamide, including hemato-cardiotoxicity, this study aimed to investigate the efficiency of quinoa ethanolic extract to ameliorate cardio-hemato-toxicity accompanied cyclophosphamide therapy in male rats. After acclimatization, adult male Wistar albino rats (160-180g) were randomly divided into four groups (10 animals each); the first group included normal rats and served as control, second group included normal rats orally administration with 400 mg/kg of quinoa ethanolic extract (QEE) for 4 weeks, third group included rats intraperitoneally intoxicated with of cyclophosphamide® (150 mg/kg/week) for a similar period, and fourth group included rats intoxicated with cyclophosphamide 4 weeks then orally ingested with QEE another four weeks. The obtained results revealed that QEE markedly restored the cardiological and hematological deteriorations induced by cyclophosphamide®; this was evidenced by the significant reduction in cardiac MDA and NO coupled with marked elevation of cardiac GSH, SOD, GPx and CAT. Also, serum CK, LDH, TNF-α, IL 1β, IL6, cholesterol, triglycerides and LDL were markedly lowered with elevated HDL. Hematological indices and hemoglobin derivatives were markedly restored. In conclusion, QEE exhibited anti-cardio-hematotoxicities property that may be mechanized through the radical scavenging and antioxidant characteristics of its active constituent's especially high phenolic content; reflecting a promising potency of QEE as cardio-hemato protective supplement.

Introduction

Many anticancer drugs exhibit potentially life-threatening effects on the immune and hematopoietic systems [1]. The treatment by mean of chemotherapy is effective for treating many cancerous tumors although it exhibits adverse effects on normal cells. In particular, this treatment may destroy hematopoietic stem cells, thereby inducing severe side effects, such as anemia and leukopenia.

Cyclophosphamide® (CPA) is perhaps the most widely used anti-neoplastic agent [2]. It is used for the treatment of chronic and acute leukemias, myelomas, lymphomas, and for bone marrow transplantation [3]. CPA also possesses a highly potent immunosuppressant activity. Apart from having tumor selective action, it also possesses many highly toxic side-effects. Dose-mediated cardiotoxicity is one of the most important toxic effects [4]. The incidence of fatal cardiomyopathy due to a single high dose of CPA is up to 17%, depending on the different regimens and patient populations [5]. In contrast to the delayed cardiotoxic effects of other anti-neoplastic drugs, CPA causes lethal cardiomyopathy within 1-10 days after first administration of the dose (180-200mg/kg) [6, 7]. The cardiotoxic effects of CPA consist of acute, dose-dependent cardiac damage, morphologically characterized by necrosis, hemorrhage and later development of fibrosis [8].

The anti-neoplastic activity of CPA is due to phosphoramide mustard, the therapeutically active metabolite, which possesses significant DNA-alkylating activity [9, 10]. The other metabolite, acrolein interferes with antioxidant system producing highly reactive oxygen-free radical superoxide radicals and hydrogen peroxide [11]. These reactive oxygen species cause damage to the inner mitochondrial membrane of the heart cells, diminishing the oxygen radical detoxifying capacity of cardiac mitochondria [12].

Natural products are known to possess wide range of biological activity. Flavonoids and polyphenolic compounds are the active antioxidant principles found in large number of natural products [13]. Quinoa

©JIPBS, All rights reserved

(*Chenopodium quinoa*) is considered as super food since it is a good source of complete protein as it contains all nine essential amino acids, unsaturated fatty acids, minerals, vitamins, fiber, and antioxidants. It is a pseudo-cereal that has been cultivated in the Andean region of South America for thousands of years and belongs to the Chenopodiaceae family [14]. Its seeds contain significant amounts of bioactive compounds, including polyphenols (mainly phenolic acids, including vanillic acid, ferulic acid, and their derivatives, as well as flavonoids, including quercetin, kampferol, and their glycosides) and tocopherols (Vitamin E), tocotrienols and carotenoids [15]. Previous studies [16] observed that bioactive compound of Quinoa could change antioxidant status in the organism by preventing oxidative stress and also helps to reduce the risk of various chronic diseases risk such as anti-inflammatory, immunomodulatory and anticarcinogenic [17]. This study aimed to explore the ameliorative effects of Quinoa ethanolic extract (QEE) against cardiotoxicity and hematopoietic toxicities complicated by cyclophosphamide® therapy.

Materials and methods

Materials

Quinoa (*Chenopodium quinoa*, Family: Amaranthaceae) seeds were obtained from Imtenan Health Shop, Industrial Area, Obour City, Cairo, Egypt. The seeds were identified by special herbalists at Basic Centre of Science, Misr University for Science and Technology, Cairo, Egypt, and the plant was found carrying a taxonomic serial number (TSN 506567).

Extraction

Quinoa seeds were washed under running tap water, air-dried, and were then powdered mechanically into fine powder. Extraction process was carried out according to the method of Miranda *et al.* [18] with slight modification. Firstly, samples were defatted twice with hexane at sample-solvent ratio of 1:5 (w/v) for 2 min by direct sonication. The mixture was then filtered through sterile filter paper (Whatman number 42) then the supernatant was discarded and the precipitate was dried and extracted with absolute ethanol at a ratio 1:10 (w/v) for 15 min by direct sonication below 40°C, then filtered under vacuum and dried using a rotary evaporator (N-1200A, Eyela, Shanghai, China) at 40°C. The resulted ethanolic extract was stored at -20°C until further use.

Determination of total yield

The filtrate was transferred to a quick fit round bottom flask with known weight (W1), freeze dried and weighted again (W2) and finally the yield calculated from the following formula:

Extract yield (g/ g crude herb) = (W2 −W1)/W3
Where,
W1 is the weight of clear and dry quick fit flask in grams,
W2 is the weight of the flask after freeze drying in grams
W3 is the weight of the crude powdered herb in grams

Determination of radical scavenging (RSA) activity by DPPH assay

Certain amount from the crude extract was dissolved in methanol to obtain a concentration of 200 ppm. A volume of 0.2 ml of this solution was completed to 4 ml by methanol; then 1 ml DPPH solution (6.09 x 10-5 mol/L, dissolved in the same solvent) was added. The absorbance of the mixture was measured at 516 nm after 10 min standing. Also, the absorbance of the reference sample or blank (1 ml of DPPH solution and 4 ml methanol) was measured. Triplicate measurements were made and the radical scavenging activity was calculated by the percentage of DPPH that was scavenged according to Nogala- Kalucka *et al.* [19].

Determination of total phenolic content

Phenolic content of the QEE was performed by dissolving five mg of the extract in 10 ml mixture of acetone and water (6:4 v/v). Then, a sample of 0.2 ml was mixed with 1.0 ml of Folin-Ciocalteu reagent (10 fold diluted) and 0.8 ml of sodium carbonate solution (7.5%). After 30 min at room temperature, the absorbance was measured at 765 nm using Cary 100 UV-Vis spectrophotometer. Estimation of phenolic compounds as catechin equivalents was carried out using standard curve of catechin [20].

Estimation of reducing power

Reducing power of the used extract was determined according to the method described by Sethiya *et al.* [21] with some modifications. From both extract and ascorbic acid, 0.5 ml of different concentrations (50, 100, 200, 400 and 800µg /ml) was mixed with 2.5 ml phosphate buffer (pH 7.4) and 2.5 ml potassium ferricyanide (0.1 M); the mixture was kept at 50ºC in a water bath for 20 minutes; then after cooling, 2.5 ml of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. Immediately, 2.5 ml of the upper layer of the solution was mixed with 2.5 ml distilled water and 0.5 ml of a freshly prepared ferric chloride solution (40% w/v). The absorbance of sample and standard was measured at 700 nm. The reducing power was calculated as equivalent to ascorbic acid from the reducing power standard curve of ascorbic acid. Control blank included mixture components without sample or standard.

Animals and experimental design

Male albino rats (160-180g) were obtained from the Animal Colony, National Research Centre, Giza, Egypt; the animals were kept in suitable plastic cages (in a good

ventilated and controlled room conditions, 25±2°C and 12h/12h light/ dark cycles) and maintained on free access to food and water for a week before starting the experiment for acclimatization; they received human care in compliance with the standard institution's criteria for the care and use of experimental animals according to ethical committee of National Research Centre (FWA 00014747); however, this study was approved by the same ethical committee. After the animals being acclimatized with experimental room conditions, they were divided randomly into four groups (10 animals each). The first group included healthy animals that fed standard diet and intraperitoneally injected with 1 ml isotonic saline without any treatments and acting as normal control, second group included healthy animals that orally administered with 400mg/kg/day of quinoa ethanolic extract (QEE) consecutively for four weeks, third group included animals that intraperitoneally injected with 150 mg/kg/week of cyclophosphamide for four weeks and acting as positive control; and fourth group include animals that intoxicated four weeks with cyclophosphamide then orally administrated daily with QEE another four weeks.

Blood and tissue sampling

At the end treatment period, rats were fasted overnight, and following anesthesia with diethyl ether, 3-5 ml blood samples were withdrawn from the retro-orbital plexus using heparinized-sterile glass capillaries. Each blood sample was divided into 2 portions: the smaller portion was taken on heparin for the determination of hematological indices and hemoglobin-derivatives, while the other portion was cool centrifuged at 3000 rpm for 15 minutes and the sera were separated and stored at -80°C until biochemical determinations as soon as possible. After blood collection, the animals were sacrificed soon, and then the heart of each animal was dissected out, washed in saline, dried, rolled in a piece of aluminum foil and stored at -80°C for homogenization and biochemical determinations. Another specimen from the heart organ was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v); the homogenate was centrifuged at 5000 rpm for 20 minutes to remove the nuclear and mitochondrial fractions; the supernatant was divided into aliquots and stored at -80°C till the determination of the oxidative stress markers in the heart tissue.

Assessment of complete blood count

Cell blood counter (full automatic –Model PCE – 210 N, Japan) was used for measuring of red blood corpuscles (RBCs) count (10^6/cm^3), Hemoglobin (Hb) content (g/dl), hematocrit (Hct) percentage, mean corpuscular volume (MCV) (fl), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/dl), platelets (PLT) count (10^3/cm^3), and total leucocytes count (TLC) count (10^3/cm^3).

Assessment of methemoglobin

Blood methemoglobin (met-Hb) was determined, as % of total Hb, in whole heparinized blood using the colorimetric method described by Evelyn and Malloy [22]; exactly 0.2ml of the heparinized blood was lysed vigorously in 10 ml of a solution containing 4ml of the freshly prepared phosphate buffer and 6ml of non-ionic detergent solution 1%. After centrifugation at 1000g for 10 minute; the clear lysate (supernatant) was divided into two equal volumes (A&B). The absorbance (D1) of the volume A was measured at 630nm, then one drop of potassium cyanide solution was added and the absorbance (D2) was measured again at 630nm after mixing. One drop of potassium ferricyanide was added to the volume (B) and the absorbance (D3) was measured at 630nm after 5 minutes, then one drop of potassium cyanide was added and the absorbance (D4) was measured at 630nm immediately after mixing. All measurements were made against the reagent blank (buffer and the non - ionic detergent in the same proportion in sample). Methemoglobin level is then calculated according to the formula below.

met-Hb (%) = [D1x D2/D3 * D4] x100

Assessment of sulf-hemoglobin

Sulf-hemoglobin (Hb-S) level was determined, as % to total Hb, in whole anti-coagulated blood using the colorimetric method described by Van Kampen and Zulstra [23]. Mixing 0.1 ml of whole blood with 10 ml of non-ionic detergent (2%) solution, and then the absorbance (A) at 620 nm was measured. After that, one drop of potassium cyanide (5%) was added and the absorbancewas recorded at 620 nm and 578 nm after 5 minutes. The percentage of S-Hb was calculated from the equation below.

S-Hb (%) = $2*[A_{620}$ (Hb-S)/A_{578} (Hb-O_2)]

Where, A_{620}(Hb-O_2) = A_{578} / conversion factor,

A_{620} (S-Hb) = A_{620} total Hb - A_{620} (Hb-O_2).

Assessment of carboxy-hemoglobin

Carboxy-hemoglobin (Hb-CO) level (as % to total Hb) was evaluated using the method described by Van Assendelft [24]. Exactly, 0.1 ml of whole blood was diluted in 20ml of ammonia (0.4ml/l) and 20mg sodium dithionite was added. Within 10 minutes, the absorbencies at 538nm and 578 nm were recorded spectrophotometrically. The Hb-Co level was calculated from the equation below.

Hb-Co (%) = [2.44× (A_{538}/A_{578})] × 2.66.

Assessment of oxy-hemoglobin (Hb-O_2)

As the four derivatives representing 100% to total Hb, therefore Hb-O_2 (%) could be calculated mathematically from the formula Hb-O_2 (%) = [100 × Met-Hb (%)×S-Hb (%)×Hb-Co (%)].

Biochemical determinations

All the biochemical measurements were carried out using Schimadzu spectrophotometer (UV–Vis 1201, Japan). Serum ALAT and ASAT activities were determined using reagent kits purchased from Human Gesell Schaft fur Biochemical und DiagnosticambH, Germany. Lipid profile was estimated using kits purchased from DiaSys Diagnostic systems GmbH Germany. Serum urea and creatinine levels were evaluated using kits obtained from Biodiagnostic, Dokki, Giza, Egypt. LDH and CK activity was assayed colorimetrically using reagent kits purchased from BioVision, South Milpitas, California, USA.

Oxidative stress markers of heart tissue

Cardiac GSH and NO levels as well as SOD, CAT and GPx activities were estimated using kits obtained from Biodiagnostic, Dokki, Giza, Egypt.

Determination of TNF-α, IL 1β and IL6

Using ELISA (DynatechMicroplate Reader Model MR 5000, 478 Bay Street, Suite A213 Midland, ON, Canada), serum TNF-α, IL 1β and IL6 concentrations were measured using rat elisa kits (SG-10057, SG-10179 and SG-10127, respectively) purchased from SinoGeneClon Biotech Co., Ltd, No.9 BoYuanRoad, YuHang District 311112, Hang Zhou, China.

Determination of heart MDA

Cardiac MDA (indirect index for lipid peroxidation) level was determined chemically according to the method described by Ruiz-Larnea *et al.* [25]. In this method, 0.5 ml heart homogenates' supernatant was added to 4.5 ml working reagent [0.8 g thiobarbeturic acid dissolved in 100 ml perchloric acid 10%, and mixed with TCA (20%) in a volume ratio 1: 3, respectively]; then in a boiling and shaking water bath, the sample-reagent mixture was placed for 20 minutes, then carried to cool at room temperature and centrifuged for 5 minutes at 3000 rpm. Finally, the absorbance of the clear pink supernatant was measured at 535 nm against reagent blank [0.5 ml distilled water + 4.5 ml working reagent]. Cardiac MDA level (nmol/g tissue) was calculated according to the formula below.

MDA (nmol/g tissue) = [{A_{535}× 10^9 / (1.56 × 10^5) × 10^3} x AD] × 10.

Where, 1.56x105 $M^{-1}L^{-1}cm^{-1}$ is MDA extinction coefficientand AD is assay dilution (10).

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using the general linear model procedure of the statistical analysis system (SAS, 1982). The significance of the differences among different treatment groups was determined by Waller-Duncan k-ratio [26]. All statements of significance were based on probability of $p \leq 0.05$.

Results

The mean values of yield amount, total phenolic content, radical scavenging activity and reducing power of quinoa ethanolic extract are shown in figures (1-2).

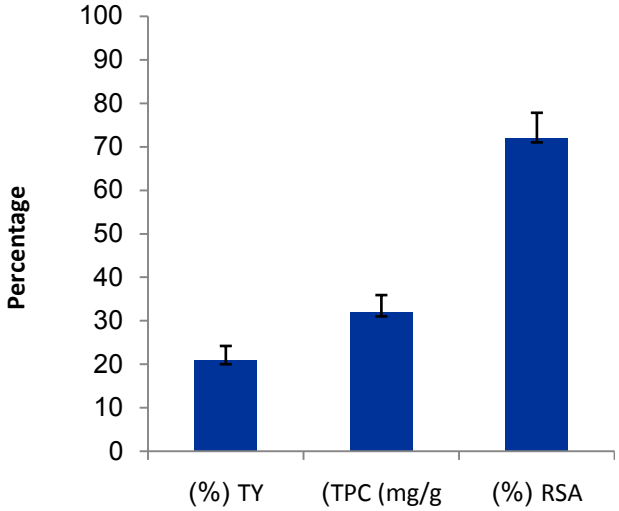


Figure 1. Yield, total phenolic content (TPC) and radical scavenging activity (RSA) of ethanolic extract of quinoa seeds.

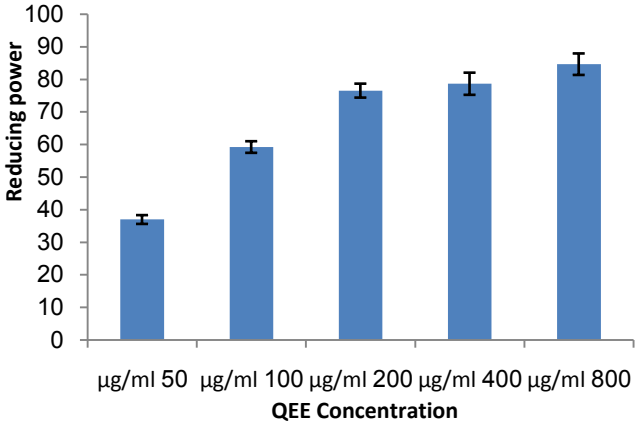


Figure 2. Reducing power of quinoa ethanolic extract.

A significant drop was noticed in Hb, HCT levels; RBC, WBCs, and platelets count; and blood indices (MCV, MCH, MCHC) as a consequence to CPA intoxication in compare to the healthy group. Favorably, administration of CPA-pretreated rats with QEE resulted in a marked improvement in the mentioned hematological parameters close to the healthy values (Table 1).

Table 1. Effect of cyclophosphamide and QEE on the level of whole blood indices.

	Control	QEE	CPA	CPA then QEE
Hb (g/dL)	13.14 ± 0.13	12.92 ± 0.17	9.5 ± 0.41*	12.6 ± 0.32#
HCT (%)	38.18 ± 1.5	39.84 ± 0.54	25.19 ± 1.04*	41.74 ± 1.2#
RBC (10^6/uL)	7.14 ± 0.37	7.62±0.24	4.84 ±0.25*	7.52 ±0.38#
MCV (fl)	53.3 ± 0.83	50.68 ± 0.53	36.63± 0.64*	56.6 ± 1.4#
MCH (pg)	17.64 ± 0.24	19.77 ± 0.27	12.4± 0.17*	17.14± 0.35#
MCHC (g/dL)	33.16 ± 0.47	32.96 ± 0.46	21.4± 0.30*	30.28± 0.38#
WBC (10^3/ uL)	7.3 ±0.54	6.42 ± 0.42	4.3± 0.33*	7.3± 0.54#
PLT (10^3/uL)	1183 ± 26.6	1200± 33.71	735± 16.5*	886± 21.2#

Data are presented as mean ±standard errorand subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. CPA is cyclophosphamide; QEE is quinoa ethanolic extract; (*) is significantly different from control group; (#) is significantly different from CPA group.

Moreover, the obtained results recorded a significant increase in the nonfunctional Hb derivatives (met-Hb, Co-Hb and S-Hb) percentage coupled with a significant drop in the functional form (Hb-O_2) in CPA group. Interestingly, post-treatment of CPA-intoxicated rats with QEE resulted in marked regulation of these nonfunctional hemoglobin derivatives downwards near those of normal group, and significantly increased the functional derivative (Hb-O_2) compared to CPA-intoxicated animals (Table 2).

Table (3) indicated that CPA intoxication induced significant elevations in serum ASAT and ALAT activities as well as urea and creatinine levels in compare to the control group. Meanwhile, post-treatment of CPA-intoxicated animals with QEE significantly restored the mentioned liver and kidney functions.

Unlikely, CPA-intoxication led to a significant increase in serum cholesterol, triglycerides and LDL level coupled with a significant decrease in HDL when compared with the control group. Interestingly, post-administration of CPA-intoxicated rats with QEE resulted in a marked improvement in lipid profile parameters monitored from the significant decrease in cholesterol, triglycerides and LDL, and the marked increase the HDL level compared to CPA-treated animals (Table 4).

In respect to Table (5), CPA-intoxication induced a marked deterioration in the cardiac oxidative stress status; evidenced by the significant increase in cardiac MDA and NO values coupled with a significant reduction in the activity of SOD, GPx and CAT as well as GSH level. In a favorable manner, QEE-treatment of animals pre-intoxicated with CPA led to a significant drop in cardiac MDA and NO levels combined with marked rise in GSH level, and SOD, CAT and GPx activities in compare to CPA-intoxicated group.

Table 2. Effect of cyclophosphamide and QEE on hemoglobin derivatives of different variants.

	Control	QEE	CPA	CPA then QEE
Hb-O_2 (%)	79.2 ± 1.32	98.1 ± 1.22	86.3 ± 1.11*	94.4 ± 1.74#
Met-Hb (%)	1.81 ± 0.21	1.17 ± 0.09	8.8 ± 0.24*	4.20 ± 0.35#
Co-Hb (%)	0.55±0.17	0.47±0.08	4.51 ±0.14*	1.06 ±0.06#
S-Hb (%)	0.24± 0.08	0.26 ± 0.02	0.39± 0.07*	0.31 ± 1.34#

Data are presented as mean ±standard error and subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. CPA is cyclophosphamide; QEE is quinoa ethanolic extract; (*) is significantly different from control group; (#) is significantly different from CPA group.

Table 3. Effect of cyclophosphamide and QEE on serum ALAT and ASAT activity as well as urea and creatinine levels.

	Control	QEE	CPA	CPA then QEE
ALAT (U/L)	86.4±4.22	85.41±3.24	279.4±6.35*	169.4±5.21#
ASAT (U/L)	130.9±4.4	124.3±4.12	463.1±7.2*	279.4±5.41#
Urea (mg/dl)	35.2±3.64	36.63±3.15	96.25±4.99*	58.85±2.89#
Creatinine (mg/dl)	0.82±0.05	0.78±0.07	2.98±0.09*	1.69±0.08#

Data are presented as mean ±standard error, and subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. CPA is cyclophosphamide; QEE is quinoa ethanolic extract; (*) is significantly different from control group; (#) is significantly different from CPA group.

Table 4. Effect of cyclophosphamide and QEE on serum cholesterol, triglycerides, LDL and HDL levels.

	Control	QEE	CPA	CPA then QEE
CHO (mg/dl)	80 ± 2.31	78 ± 4.22	321 ± 9.65*	163 ± 6.66#
TRG (mg/dl)	72 ± 5.92	70 ± 5.23	258 ± 10.22*	124 ± 9.33#
LDL-C (mg/dl)	42±3.66	43±5.54	188 ±7.54*	165±6.65#
HDL-C (mg/dl)	38± 1.45	40 ± 2.16	19 ± 3.25*	32 ± 1.34#

Data are presented as mean ±standard error, and subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. CPA is cyclophosphamide; QEE is quinoa ethanolic extract; (*) is significantly different from control group; (#) is significantly different from CPA group.

Table 5. Effect of cyclophosphamide and QEE on cardiac MDA, NO, GSH, SOD, CAT and GPx.

Oxidative stress	Control	QEE	CPA	CPA then QEE
MDA (µmol/g tissue)	1.22±0.31	1.26±0.24	2.48±0.11*	1.45±0.14#
NO (µmol/g tissue)	340±21	352±27	572±38*	403±29#
GSH (nmol/g tissue)	14.52±1.1	15.02±2.3	9.52±1.7*	11.35±1.6#
SOD (U/g tissue)	1598±58	1622±61	924±52*	1266±66#
CAT (U/g tissue)	4.47±0.53	5.02±0.48	2.62±0.36*	3.79±0.24#
GPx(U/g tissue)	27.6±1.03	26.5±1.12	14.4±1.08*	21.8±1.43#

Data are presented as mean ±standard error, and subjected to one-way ANOVA followed by post hoc test (Duncan) at $p \leq 0.05$. CPA is cyclophosphamide; QEE is quinoa ethanolic extract; (*) is significantly different from control group; (#) is significantly different from CPA group.

The obtained data showed that a significant increase in serum CK and LDH activity in CPA group when compared with the control group. Interestingly, administration of CPA-intoxicated rats with QEE improved serum CK and LDH activity within normal values as well as it significantly decreased TNF-α, compare to CPA-intoxicated animals' group (figure 3).

Figure (4) showed that treatment with CPA displayed a significant increase in serum TNF-α, IL1β and IL6 level when compared with the control group. However, post-administration of CPA-intoxicated rats with QEE resulted in marked improvement in level of the measured inflammatory cytokines (TNF-α, IL1β and IL6) compared to CPA-intoxicated animal.

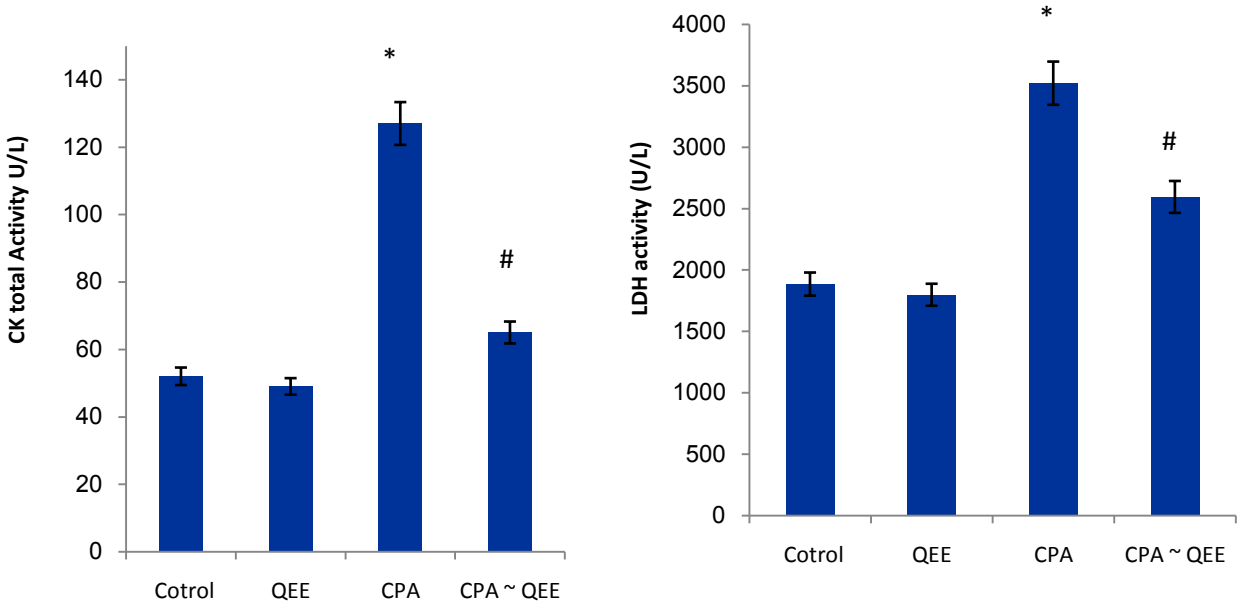


Figure 3. Serum CK and LDH activity of control, CPA ®-intoxicated and QEE -treated male albino rats. * is significantly different from control group, while # is significantly different from CPA group ($p \leq 0.05$).

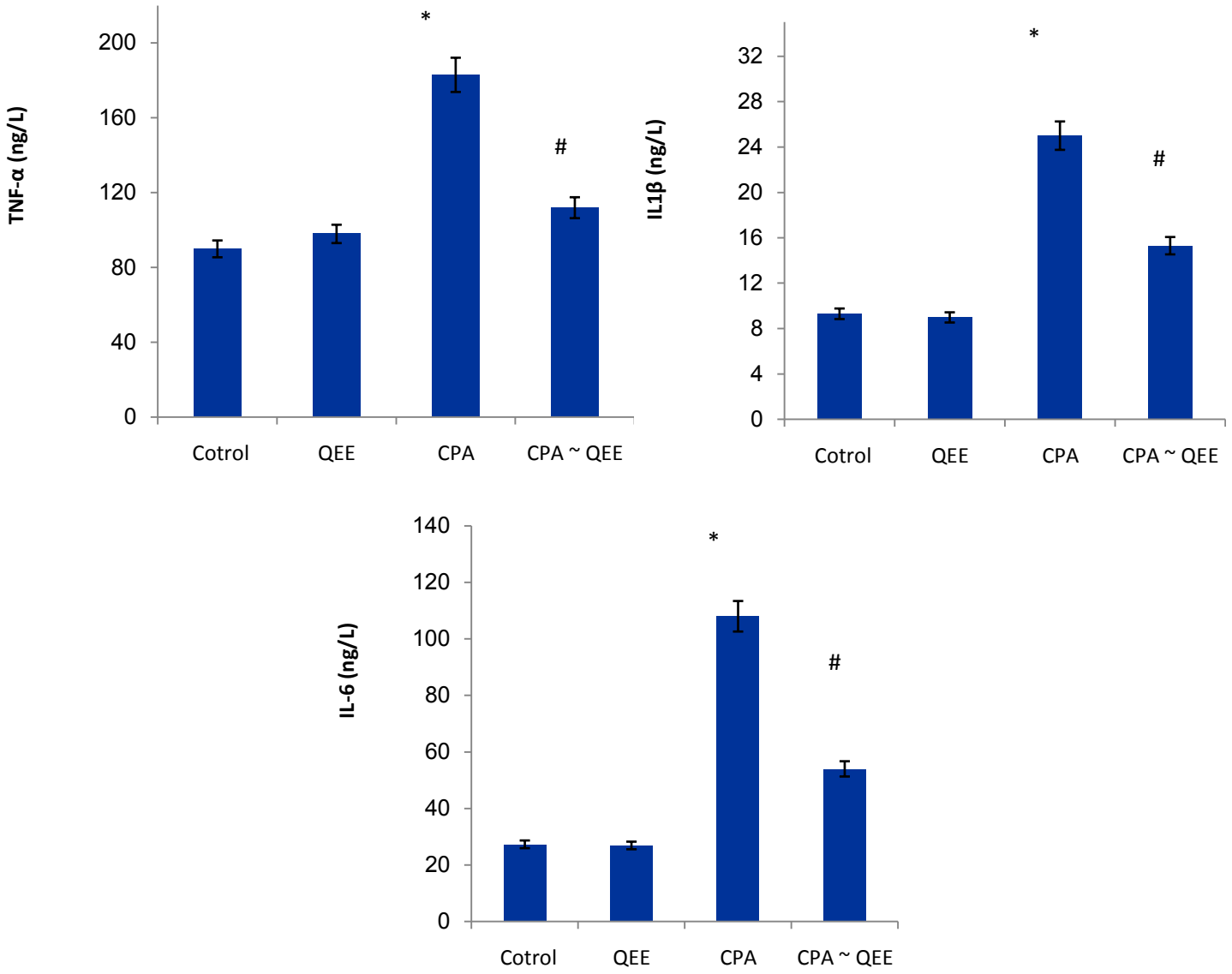


Figure 5. Serum TNF-α, IL1β and IL-6 levels of control, CPA ®-intoxicated and QEE -treated male albino rats. (*) is significantly different from control group; (#) is significantly different from CPA group (p≤ 0.05).

Discussion

Cyclophosphamide® (CPA) is a cytotoxic drug that is highly effective in the treatment of various human cancers particularly lymphomas and some types of leukemia and autoimmune diseases. The clinical utility of the oncolytic agent has been hampered by dose-limiting toxicities; one of the most frequent complications is myocarditis [27]. This study aimed to investigate the ameliorating potential of QEE on hemato-cardiotoxicities accumulated as consequence to CPA-therapy in rats.

In the present study, CPA administration significantly increased the activities of serum LDH, CK, ASAT and ALAT; this result is in accordance with the studies of Bhatt *et al.* [28] and Omole *et al.* [29]. It was stated that CPA is a cardiotoxic agent inducing a direct myocardial endothelial damage and destruction of myocardial cells; thus LDH, CK and ASAT were raised. Similarly, CPA is a hepatotoxic chemical that metabolized into a toxic metabolite (acrolein) that could deteriorate the cellular-integrity and membrane-permeability of hepatocytes leading to leakage of hepatic ASAT and ALAT to blood stream [30]. These pathophysiological changes might be due to the overproduction of reactive oxygen species during CPA-treatment, which cause membrane injury by triggering the production of lipid peroxidation that result in loss of function and integrity of myocardial and hepatocytes membranes [31]. Thus, this effect might be due to the CPA toxic metabolite; acrolein [32].

It was reported that Quinoa seeds contain considerably high vitamin E, iron, zinc and magnesium as well as phytosteroids [33]. Also, Abugoch [34] stated that there are antioxidant compounds such as polyphenols, phytosterols and flavonoids in quinoa seeds; these

substances may be related with these effects herein. Matsuo [35] demonstrated that the use of QEE can also be beneficial for increasing the production of cardiac antioxidant enzymes. This exhibition is related with the reduction of harmful effects caused by free radicals on the human body, which it leads to a reduced endothelial alterations (endothelial dysfunction) and decreased oxidation of LDL-c molecules, and hence, reduces the risks for vascular diseases [36].

Intoxication with CPA led to significant deteriorations in all hematological measurements monitored from the reduced RBCs count, hematocrit percentage, MCV, MCH and MCHC leading to a condition of hypochromic-microcytic anemia that resulted in a dependent degree of tissue hypoxia, especially heart and brain tissues. This was in accordance with Stalin and Kumar [37] who reported that CPA exhibiting adverse-effects on blood and bone marrow due to interaction of its active metabolites with the hematopoietic tissues which subsequently cause depression of the hematopoietic activities. Furthermore, CPA caused decrease in these measurements due to immunosuppressive effect of CPA and retarded haematopoeisis and /or interaction of CPA active metabolic with proteins of the erythrocytes membranes leading to elevation of RBCs destruction rate and decrease in Hb synthesis; hence decline in MCV (microcytosis), MCH and MCHC (hypochromasia) [38] resulting finally in a case of hypochromic-microcytic anemia which mainly a manifestation of iron-deficiency. It could seated that CPA may inhibit either absorption or assimilation of iron.

With respect to the results of hemoglobin derivatives of different ligands, CPA intoxication resulted in a significant increase in the non-functional hemoglobin derivatives (met-Hb, S-HB and Co-Hb) coupled with a marked drop in the functional derivative ($Hb\text{-}O_2$) indicating that CPA induced oxidative stress on red blood cells.

It was stated that free radicals attack microsomal lipids leading to their peroxidation and also covalently bind to microsomal lipids and proteins; this leads to generation of reactive oxygen species (ROS), including superoxide anion $O^{\bullet -2}$, H_2O_2 and the hydroxyl radical [39, 40].

Direct exposure to molecular oxygen and circulating components in the blood and the loss of the de novo synthesizing capacity of new enzyme molecules during maturation put the erythrocytes at high risk of damage by superoxide anion and H_2O_2 molecules. These reactive molecules are involved in lipid peroxidation [41], the oxidation of thiol-groups of enzymes [42] and the oxidative degradation and denaturation of Hb [43]. The oxidized hemoglobin (met-Hb) then precipitates and covalently binds to the interior erythrocytes membrane, forming Heinz bodies that distorts the cell membrane and results in increased erythrocytes fragility and hemolysis [44]. Also, CPA significantly affects the quantity and function of Hb molecule whereas total Hb and Oxy-Hb contents decreased significantly concomitant with significant increases in Met-Hb, Sulf-Hb and Carboxy-Hb contents. These changes may, therefore, is a consequence of the increase in oxidative stress which caused by free radicals generated during the metabolic degradation of CPA. Erythrocytes under oxidative stress, there is a considerable rise in the level of Met-Hb, which is known to be incapable of reversible oxygen binding [45]. Met-Hb is formed when the ferrous porphyrin complex of Hb is oxidized into the ferric form [46]. *In vivo*, Met-Hb is predominately reduced by the NADH cytochrome b5-Met-Hb reductase system, and minor pathway such as the NADPH-dependent Met-Hb reductase [47]. It was suggested that NADPH concentration may be important in preventing Met-Hb generation. Loss of NADPH and glutathione (GSH) are thought to account for the enhanced rates of Met-Hb generation and lipid peroxidation [48]. The free radicals may also induce conformational changes in Hb molecule and make it susceptible to bind unfavorable ligands other than oxygen such as carbon monoxide (CO) and sulphur (S). QEE improved the hemoglobin function, quantitatively and qualitatively, since it could elevated total Hb and Oxy-Hb contents and reduced met-Hb, S-Hb and Co-Hb level. These data further confirm the antioxidant potential of QEE. This may in turn improves liver function and consequently improves the synthesis of the antioxidant enzymes and the reduced glutathione required for erythrocytes protection. A correlation analysis previously demonstrated a strong relation between the healthy state of the liver and erythrocytes function; our study found a significant negative correlation between the blood indices and cardiac MDA and NO level, while positive correlation with the SOD, CAT, GPx and GSH. Total Hb and Oxy-Hb contents also correlated negatively while Met-Hb, S-HB and Carboxy-Hb correlated positively with the cardiac MDA level (data unpublished). These results are in accordance with Abdel-Wahhab *et al.* [49]. The anti-hematotoxic effect of QEE against CPA-induced hematotoxicity was significantly observed as it succeeded in restoring the blood indices close to its control levels. QEE is rich in various polyphenolic compounds [50]; presence of these polyphenols contributes significant role in improving the antioxidant voltage of the body against CPA active metabolites and the liberated radicals as well; thereby reversing/ameliorating the anemic condition induced by CPA. This might refer to reversal of bone marrow depression thus improving hematopoeitic activity of the cells. It improved the integrity of erythrocyte membranes through the antioxidant potential, thereby reducing hemolysis. In addition, the quinoa extract may enhance protein synthesis which leads to increase in Hb level and consequently results to improve MCV, MCH and MCHC values and RBC.

In addition, intoxication with CPA recorded a significant decrease in the antioxidant battery, evidenced by the drop in SOD, CAT, GPx and GSH values, giving multi-chances to cause membrane-injury which resulted in increased level of the oxidative markers (MDA and NO); leading to loss of function and integrity of myocardial membrane [51]; excess free radical generation mediated by lipid peroxidation causes increased glutathione consumption. The marked decrease in SOD, CAT, GPx and GSH values promotes the formation of hydroxyl radicals, and initiation and propagation of lipid peroxidation. However, it is suggested that the decrease in the activities of these antioxidant markers is a consequence of increased oxidative stress in the cardiac tissues due to the overproduction of active reactive oxygen species [52].

Treatment of CPA-intoxicated animals with QEE restored level of GSH and the antioxidant battery (SOD, CAT, and GPx) and reduced the levels of oxidative markers (MDA and NO) in the cardiac tissues near those of control ones, indicating a ameliorating effect of QEE against CPA-mediated reactive oxygen species. This effect might be due to QEE-ability to decrease oxidative stress and preserve the activity of antioxidant enzymes as well as its ability to inhibit lipid peroxidation hydroxyl radical [53]. Another possible mechanism could be considered that is the possible QEE ability to correct deficient thiol status of the cardiac cells by increasing the synthesis of GSH. Also, QEE has a strong antioxidant effect, as it may decrease lipid peroxidation in the heart tissue and prevent free radicals induced-damage to the myocardium by its free radical scavenging effect that possibly mechanized through polyphenols, phytosterols, and flavonoids in QEE. [54]. It was demonstrated that GSH has a potential effect to reduce CPA-induced lipid peroxidation and also increase therapeutic index of the drug by way of reducing its toxicity that may be mediated through free radical mechanisms [55].

Proinflammatory cytokines reflect the degree of inflammation. CPA administration induced significant increases in serum TNF-a, IL- 1β, IL-6 levels. This finding is similar to that recently reported by Lixin [56]. The elevated levels of circulatory inflammatory cytokines are in consistent with the recorded significant up-regulation of NF-KB, induced by ROS, which is well known to regulate the expression of various genes including inflammatory cytokines and iNOS [57]. CPA promoted up-regulation of NF-kB and iNOS with subsequent production of NO. Treatment of rats with QEE potentially ameliorated CPA-induced-inflammation through down regulation of NF-$_K$B and iNOS expression, and attenuation of pro-inflammatory cytokines production. This interesting exhibition provides strong evidence on the anti-inflammatory efficacy of QEE, which is one of the significant mechanisms for reduction of drug-induced cardio-hematotoxicity. The anti-inflammatory behavior of QEE may be mechanized through the considerably high vitamin E, iron, zinc and magnesium contents [33]; saponins, polyphenols, flavonoids and phytosteroids [34] related with the effects of reduction of inflammation.

Conclusion

Administration of QEE post-CPA intoxication succeeded in amelioration of the hematopoeitic and cardiac induced-deteriorations. This exhibition might be mechanized by free radical scavenging, increased activity of the antioxidant defense systems, and membrane stabilizing properties due the antioxidant constituents present in QEE. As QEE has been used extensively as an additive therapy and traditional medicine for several diseases, it could be a potential candidate for a safe supplemental agent against xenobiotic toxicity.

References

1. Ryu HS, Kim JH, and Kim HS. Effect of Plant Water Extract Mixture (Ixeris sonchifolia Hance (Ish), Oenanthe javanica (Oj), Fagopyrum esculentum Moench (Fem), Hizikia fusiforme (Hf) and Zingiber officinale Roscoe (Zor) on Mouse and the Modulation of Immune Cell Activation. The FASEB journal 2007; 21 (5): A375-A375.
2. Gershwin ME, Goetzl EJ, Steinberg AD. Cyclophosphamide: use in practice. Ann Intern Med 1974; 80:531e40.
3. Goldberg MA, Antin JH, Guinan EC, Rappeport JM. Cyclophosphamide cardiotoxicity: an analysis of dosing as a risk factor. Blood 1986; 68:1114e8.
4. Fraiser LH, Kanekal S, Kehrer JP. Cyclophosphamide toxicity: characterizing and avoiding the problem. Drugs 1991; 42:781e95.
5. Taniguchi I. Clinical Significance of cyclophosphamide-induced cardiotoxicity. Intern Med 2005; 44:89e90.
6. Gottdiener JS, Appelbaum FR, Ferrans VJ, Deisseroth A, Ziegler J. Cardiotoxicity associated with high-dose cyclophosphamide therapy. Arch Intern Med 1981; 141:758e63.
7. Braverman AC, Antin JH, Plappert MT, Cook EF, Lee RT. Cyclophosphamide cardiotoxicity in bone marrow transplantation: a prospective evaluation of new dosing regimens. J. Clin. Oncol. 1991; 9:1215e23.
8. Mills BA, Roberts RW. Cyclophosphamide-induced cardiomyopathy. A report of two cases and review of the English literature. Cancer 1979; 43: 2223e6.
9. Cushnir JR, Naylor S, Lamb JH, Farmer PB, Brown NA, Mirkes PE. Identification of phosphoramide mustard/DNA adducts using tandem mass spectrometry. Rapid Commun Mass Spectrom. 1990; 4:410e4.
10. Povirk LF, Shuker DE. DNA damage and mutagenesis induced by nitrogen mustards. Mutat. Res. Genet. Toxicol. 1994; 318:205e26.
11. Clarke L, Waxman DJ. Oxidative metabolism of cyclophosphamide: identification of the hepatic monooxygenase catalysts of drug activation. Cancer Res 1989; 49:2344e50.
12. Souid AK, Tacka KA, Galvan KA, Penefsky HS. Immediate effects of anticancer drugs on mitochondrial oxygen consumption. Biochem Pharmacol. 2003; 66: 977e87.
13. Lin T, Ke B, Cheng CS, Wang J, Wei BL and Lee CL. Red Quinoa Bran Extracts Protects against Carbon Tetrachloride-Induced Liver Injury and Fibrosis in Mice via Activation of Antioxidative Enzyme Systems and Blocking TGF-1 Pathway Nutrients 2019; 11:395.
14. Jancurová M, Minarovicová L, Dandar A. Quinoa–a review. Czech J Food Sci. 2009; 27:71-9.
15. Viswanatha SAH, Patel UM, Koti BC, Gadad PC, Patel NL, Thippeswamy AH. Cardioprotective effect of Saracaindica against cyclophosphamide-induced cardiotoxicity in rats: a biochemical, electrocardiographic and histopathological study. Indian J Pharmacol. 2013; 45:44-48.
16. Laus MN, Cataldi MP, Robbe C, D'Ambrosio T, Amodio ML, Colelli G, *et al.* Antioxidant capacity, phenolic and vitamin C contents of quinoa (Chenopodium quinoa Willd.) as affected by sprouting and storage conditions. Ital. J. Agron. 2017; 12:816.

17. Pasko P, Barton H, Zagrodzki P, Izewska A, Krosniak M, Gawlik M, *et al.* Effect of diet supplemented with quinoa seeds on oxidative status in plasma and selected tissues of high fructose-fed rats. Plant Foods Hum Nutr. 2010; 65(2):146-51.
18. Miranda M, Delatorre-Herrera J, Vega-Gálvez A, Jorquera E, Quispe-Fuentes I, Martínez EA. Antimicrobial potential and phytochemical content of six diverse sources of quinoa seeds (Chenopodium quinoa Willd.) Agric Sci. 2014; 5:1015–1024.
19. Nogala-Kalucka MJ, Korczak M, Dratwia E, Lampart-Szczapa A, Siger, and Buchowski M. Changes in antioxidant activity and free radical scavenging potential of rosemary extract and tocopherols in isolated rapeseed oil triacylglycerols during accelerated tests. Food Chemistry 2005; 93: 227–235.
20. Jayaprakasha GK, Selvi T, Sakariah KK. Antibacterial and antioxidant activities of grape (Vitisvinifera) seed extracts. Food Research International 2003; 36(2):117-122.
21. Sethiya NK, Trivedi A, Mishra S. The total antioxidant content and radical scavenging investigation on 17 phytochemical from dietary plant sources used globally as functional food. Biomed Prev. Nutr. 2014; 4:439–444. doi: 10.1016/j.bionut.2014.03.007.
22. Evelyn KA and Malloy HT. Micro determination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. Journal of Biological chemistry 1983; 126: 655-660.
23. Van kampen EJand Julstra WG. Determination of hemoglobin and its derivatives. Advances in Clinical Chemistry 1965; 8: 141 - 149.
24. Van Assendelft OW. Spectrophotometry of Haemoglobin Derivatives. Ann Intern Med. 1970; 75(1):150-151.
25. Ruiz-Larnea MB, Leal AM, Liza M, Lacort M and Groot H. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron induced lipid peroxidation of rat liver microsome. Steroid. 1994; 59: 383–388.
26. Waller RA and Duncan DB A. Bayes rule for the symmetric multiple comparison problems. J Am Stat Assoc. 1969; 64:1484–1503.
27. Hu RQ, Mehter H, Nadasdy T, Satoskar A, Spetie DN and Rovin BH. Severe hemorrhagic cystitis associated with prolonged oral cyclophosphamide therapy: case report and literature review. Rheumatol Int. 2008; 28:1161–4.
28. Bhatt L, Sebastian B and Joshi V. Mangiferin protects rat myocardial tissue against cyclophosphamide induced cardiotoxicity Mangiferin protects rat myocardial tissue against cyclophosphamide induced cardiotoxicity. Journal of Ayurveda and Integrative Medicine 2017; 8: 62-67.
29. Omole JG, Ayoka OA, Alabi QK, Adefisayo MA, Asafa MA, Olubunmi BO, and Fadeyi, BA. Protective Effect of Kolaviron on Cyclophosphamide -Induced Cardiac Toxicity in Rats. Journal of Evidence-Based Integrative Medicine 2018; 23: 1-11.
30. Viswanatha SAH, Patel UM, Koti BC, Gadad PC, Patel NL, Thippeswamy AH. Cardioprotective effect of Saracaindica against cyclophosphamide-induced cardiotoxicity in rats: a biochemical, electrocardiographic and histopathological study. Indian J Pharmacol. 2013; 45:44-48.
31. Chakraborty P, HossainSk U, Murmu N, Das JK, Pal S, Bhattacharya S. Modulation of cyclophosphamide-induced cellular toxicity by diphenyl-methyl selenocyanate In vivo, an enzymatic study. J. Cancer Mol. 2009; 4:183-189.
32. Senthilkumar S, Devaki T, Manohar BM, Babu M.S. Effect of squalene on cyclophosphamide induced toxicity. Clin. Chim. Acta. 2006; 364:335-342.
33. Kuljanabhagavad T. *et al.* Triterpene saponins from Chenopodium quinoa Willd. Phytochemistry 2008; 69(9):1919-26.
34. Abugoch, and LE. Chapter 1 Quinoa (Chenopodium quinoa Willd.) Composition, Chemistry, Nutritional Functional Properties. Advances in Food and Nutrition Research 2009; 58: 1-31.
35. Matsuo M. In vivo antioxidant activity of methanol extract from quinoa fermented with Rhizopusoligosporus. Journal of Nutrition Science and Vitaminology 2005; 51(6):449-52.
36. Adler A. *et al.* Investigation of the Use of Antioxidants to Diminish the Adverse Effects of Postnatal Glucocorticoid Treatment on Mortality and Cardiac Development. Neonatology 2010; 98(1):73-83.
37. Stalin S, Kumar PS. Immunostimulatory Effect of Aqueous Extract of Leucasaspera in Cyclophosphamide Induced Immunosuppressive Mice. International Journal of Pharm Tech Research 2013; 5(3):910-914.
38. Promise N, Agomuo EN, Uloneme GC, Egwurugwu JN, Omeh YN, Nwakwuo GC. Effect of Phyllanthusamarus leaf extract on alterations of haematological parameters in Salmonellae typhi infested wistar albino rats. Scientific Research and Essays. 2014; 9(1):7-12.
39. Packer JE, Slater TF and Willson RL. Reactions of the carbon tetrachlonde-related peroxy free radical with amino acids: pulse radiolysis evidence. Life Sci. 1978; 23: 2617-2620.
40. El Denshary ES, Al-Gahazali MA, Mannaa FA, Salem HS, Hassan NS and Abdel-Wahhab MA. Dietary honey and ginseng protect against carbon tetrachloride-induced hepatonephrotoxicity in rats. Exp. Toxicol. Pathol. 2012; 64: 753-760.
41. GadAS, Khadrawy YA, El-Nekeety AA, Mohamed SR, Hassan NS, Abdel-Wahhab MA. Antioxidant activity and hepatoprotective effects of whey protein and Spirulina in rats. Nutr. 2011; 5: 582-589.
42. Jacob HS and Jandl H. Effects of sulfhydryl inhibition on red blood cells. II. Studies in vivo. J. Clin. Invest. 1962; 14: 1514-1523.
43. Carrel RW, Winterboum CC, Rachmilewitz EA. Activated oxygen and hemolysis. Br. J. Hematol. 1975; 30: 259-264.
44. Rae HA. Onion toxicosis in a herd of beef cows. Can. Vet. J. 1999; 40: 55-57.
45. Lukyanenko LM, Kozlova NM, Slobozhanina EI. Activity of membrane-bound NADH methemoglobin reductase and physical state of lipids in erythrocyte membranes. Bioelectrochem. 2004; 62: 191-193.
46. Jaffe ER. Cytochrome b5 reductase deficiency and enzymopenic hereditary methemoglobinemia. "The metabolic and molecular basis of inherited diseases", 6th edn. McGraw Hill Information Services Company, New York, pp: 1995; 2269-2280.
47. Kennett EC, Ogawa E, Agar NS, Godwin IR, Bubb WA and Kuchel PW. Investigation of methaemoglobin reduction by extracellular NADH in mammalian erythrocytes. Int. J. Biochem. Cell Biol. 2005; 37: 1438-1445.
48. Makni M, Chtourou Y, Fetoui H, Garoui M, Barkallah M, MarouaniC, Kallel C and Zeghal N. Erythrocyte oxidative damage in rat treated with CCl4: protective role of vanillin. Toxicol. Ind. Health 2012; 28(10): 908-916.
49. Abdel-Wahhab KG, Mannaa FA and Abdel-Wahhab MA. The protective effects of whey protein and Spirulina against CCl4-induced erythrocyte damage in rats. Journal of Applied Sciences Research 2013; 9(3): 2063-2071.
50. Hejazi MA. Preparation of different formulae from quinoa and different sources dietary fiber to treat obesity in rats. Nature and Science 2016; 14 (2): 55-65.
51. Hamon NW. Hawthorns: the genus crataegus. Can Pharm J. 1996;: 121708-709.
52. Jnaneshwari S, Hemshekhar M, Santhosh MS, et al. Crocin, a dietary colorant mitigates cyclophosphamide-induced organ toxicity by modulating antioxidant status and inflammatory cytokines. J Pharm Pharmacol. 2013; 65:604-614.
53. Li L, Lietz G, Bal W, Watson A, Morfey B and C Seal. Effects of Quinoa (Chenopodiumquinoa Willd.) Consumption on Markers of CVD Risk. Nutrients 2018; 10: 777
54. Konishi, Y, Hirano S, Tsuboi H and Wada M. Distribution of minerals in quinoa (Chenopodium quinoa willd.) seeds. Biosci. Biotechnol. Biochem. 2004; 68: 231–234.
55. Ray S, Pandit B, Ray SD, Das S, Chakraborty S. Cyclophosphamide induced lipid peroxidation and changes in cholesterol content: protective role of reduced glutathione. Int J Pharmtech Res. 2010; 2:704-718.
56. Lixin X, Lijun Y and Songping H. Ganoderic acid A against cyclophosphamide-induced hepatic toxicity in mice J Biochem Mol Toxicol. 2019; 33:e2227.
57. Pikarsky E, Porat RM, Stein I. NF-jB functions as a tumour promoter in inflammation-associated cancer. Nature 2004; 431:461–466.