



Research Article

Mitigating Role of Quercetin Against Cyclophosphamide-Induced Lung Injury in Rats

Nora A.Ashry, Nariman M.Gameil, and Ghada M.Suddek*

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Egypt.

ARTICLE INFO

Received 12/11/2012

Received in revised form 02/03/2013

Accepted 03/03/2013

Available online 30/07/2013

ABSTRACT

Quercetin (Qur), a polyphenolic flavonoid compound present in large amounts in vegetables and fruits, plays important roles in human health through its antioxidant activity. This study was conducted to investigate the possible modulatory effect of Qur against cyclophosphamide (CP)-induced lung oxidative damage and to highlight the underlying mechanisms of such effect. Male Sprague-Dawley rats were divided into four groups. Group I was control. Group II received Qur (100 mg/kg/d. p.o.) for 14 consecutive days. Group III was injected once with CP (150 mg/kg, i.p.). Group IV received Qur for 7 consecutive days, before and after CP injection.

A single i.p. injection of CP markedly increased the level of serum biomarkers; total protein, LDH. Cyclophosphamide significantly increased the lung content of lipid peroxides and decreased levels of reduced glutathione. Treatment of rats with Qur for 7 days prior to and 7 days after cyclophosphamide significantly ameliorated the alterations in lung and serum biomarkers associated with inflammatory reactions. Moreover, Qur attenuated the secretion of pro-inflammatory cytokine, TNF- α in rat serum. In addition, Qur slightly ameliorated CP-induced histopathological changes in lung tissue.

Our results suggest that Qur produces a protective effect against CP-induced lung injury and suggest a role of oxidative stress and inflammation in the pathogenesis.

Keywords: cyclophosphamide, quercetin, tumor-necrosis factor- α , lung, rats.

1. Introduction

Cyclophosphamide (CP), an antineoplastic and immunosuppressive drug has been widely used in the acute treatment of various neoplastic diseases. Cyclophosphamide is well known to cause various histopathologic patterns of lung injury (Kim et al., 2012). Early histologic findings usually demonstrate endothelial damage, granular pneumocyte injury, pulmonary edema, hemorrhage and cellular inflammatory infiltration (Kaneke et al., 1992; Venkatesan and Chandrakasan, 1995). Although the mechanism(s) of CP-mediated lung injury is incompletely understood, generation of reactive

oxygen species and lipid peroxidation in lung microsomes are thought to be involved. Several classes of antioxidant dietary compounds have been suggested to have health benefits. The evidence shows that the consumption of these products leads to decreases in various proinflammatory and/or oxidative stress biomarkers (Vouldoukis et al., 2004). Quercetin (Qur), is a flavonoid, which is present in fruits, vegetables, and several other dietary sources (Pawlikowska-Pawlega et al., 2003). It is marketed as a diet supplement with antihistaminic, anti-inflammatory, antiviral, immunomodulatory, and antioxidant properties (Ross and Kasum, 2002). In the present study, we aimed to

*Corresponding author: ghmsuddek@yahoo.com

investigate the possible protective effect of quercetin on the antioxidative system and inflammatory cytokines in the lung after CP therapy.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats weighing 170-280 g were used in all of the experiments. They were obtained from Urology and Nephrology Center of Mansoura University, Mansoura, Egypt. The animals were maintained under standard conditions of temperature $24 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ relative humidity with regular 12 h light/12 h dark cycles. They were allowed free access to standard laboratory food and water. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee of Faculty of Pharmacy, Mansoura University, Egypt in accordance with "Principles of Laboratory Animal Care" (NIH publication No.85-23, revised 1985).

2.2. Drugs and Chemicals

Cyclophosphamide was obtained as pharmaceutical drug (Endoxan vial 200 mg). Quercetin was obtained from (Sigma Chemical Company St. Louis, MO, USA). All other chemicals used in this study were of fine analytical grade.

2.3. Experimental protocol

The animals were divided at random into four groups of 8 rats each. The first group (control) received vehicle used for Qur (CMC). The second group, received Qur (100 mg/kg/d. p.o.) for 14 consecutive days. The third group was injected once with CP (150 mg/kg, i.p.). The fourth group received Qur for 7 consecutive days, before and after CP injection. In this group, CP (150 mg/kg, i.p.) was injected once on day 7, 1 h after Qur administration. On the seventh day following CP injection, blood samples were taken under light ether anesthesia. Serum was separated by centrifugation for 5 min at 4000 rpm and stored at -20°C until TNF- α assay. Fresh serum was used to estimate total protein level and LDH activity. All rats were weighed and then killed by an overdose of ether. The lungs were isolated, washed with chilled 1.15% KCl (pH 7.4). The left lungs were used for determination of thiobarbituric acid-reactive substances (TBARS) levels, reduced glutathione contents (GSH) and superoxide dismutase activities (SOD). The right lungs were fixed in 10% neutral buffered formalin for histopathological examinations.

2.4. Biochemical Estimation

2.4.1. Estimation of serum total protein and LDH

These estimations were done according to the standard procedures given along with the kits purchased. Kits from BioMed-Diagnostics, Egy-Chem Co., Egypt were used. Estimation of serum total protein depends on the reaction of protein with copper ions (ii) to produce a blue violet color compound in alkaline medium. The color intensity is proportional to the concentration of total protein present in the sample.

Detection of serum LDH depends on the conversion of pyruvate to lactate by LDH consuming NADH⁺, which absorbs light at 340 nm. Its consumption is directly proportional to serum LDH concentration. LDH activity was calculated as units per litre.

2.4.2. ELISA detection of serum TNF- α level

Enzyme-linked immunosorbent assays (ELISAs) were used to detect TNF- α level in rat serum according to the manufacturer's manual (Quantikine R&D system Inc, Minneapolis, MN). All TNF- α determinations were performed in duplicate serial dilutions. Absorbance was read on a microplate reader and the concentrations were calculated from the standard curve.

2.4.3. Preparation of lung homogenate

The isolated left lungs were rinsed in chilled 1.15% KCl (pH 7.4) and weighed quickly. Subsequently, the lung/body weight ratio was determined. Homogenization was carried out in ice-cold KCl (1.15%, pH 7.4) to yield 10% w/v tissue homogenates (Daba et al., 2002) and the following biochemical parameters were assessed.

2.4.4. Determination of Lipid peroxidation (LP)

The level of LP in the lung was estimated as TBARS according to Ohkawa et al. (1979). The absorbance was determined at 532 nm spectrophotometrically and the concentrations were expressed as nmol/g wet tissue.

2.4.5. Determination of reduced glutathione (GSH)

The level of acid-soluble thiols, mainly GSH, in the lung was assayed colourmetrically, based on its reaction with Ellman's reagent according to the method earlier described by Ellman (1959). The absorbance was measured at 412 nm and the concentrations were expressed $\mu\text{mol/g}$ wet tissue.

2.4.6. Determination of superoxide dismutase (SOD)

The enzymatic activity of SOD was assessed according to Marklund (1985). SOD activity was expressed as U/g wet tissue. One unit of SOD activity is defined as the amount of the enzyme causing 50% inhibition of auto-oxidation of pyrogallol.

2.5. Histopathological analysis

The right lungs were rapidly removed from the animal, sliced transversely, paraffin embedded, and prepared as $3\mu\text{m}$ thickness sections stained with hematoxylin and eosin (H&E) for light microscopic evaluation. Histopathological examination of the lung sections was performed without knowledge of the treatment protocol, as described previously.

2.6. Statistical analysis

Data are expressed as mean \pm S.E.M. (Significance was calculated at $p < 0.05$). Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test

(Daniel, 1991). Also paired Student's t-test was used as a test of significance for comparison between two arithmetic means of the same subject before and after treatment (Daniel, 1991). Statistical calculations were carried out using Instat-2 computer program (GraphPad Software Inc. V2.04, San Diego, CA, USA).

3. Results

3.1. Effect of CP and/or Qur on body weight and lung/body weight ratio

The average body weight of normal control animals increased significantly after 14 days (20.6% increase). Administration of CP alone induced a significant

decrease in the average body weight (9.5% reduction). Qur-treated group showed a similar increase as in the control group. Animals treated with CP and Qur showed a significant decrease in their body weight by 7.4% which is non-significantly different from CP-treated animals (Table 1).

CP administration induced a significant increase in lung/body weight ratio of the rats compared to control non-treated animals. Combination of Qur with CP showed a significant decrease in lung/body weight ratio of the rats when compared to CP treated group (Table 1).

Table 1. Effect of cyclophosphamide (CP,150 mg/kg) and/or quercetin (Qur, 100 mg/kg) on body weight and lung/body weight ratio of rats

Treatment	Body weight (g)			Lung/body weight ratio ($\times 10^{-3}$)
	Before treatment	After treatment	% Change in body weight	
Control	179.9 \pm 6.241	217.5 \pm 6.439 ^o	20.9 \uparrow	6.4 \pm 0.2
CP	182.1 \pm 5.111	164.9 \pm 5.097 ^o	9.46 \downarrow	7.9 \pm 0.3*
Qur	177.8 \pm 6.62	210.6 \pm 5.754 ^o	18.4 \uparrow	6.8 \pm 0.3#
CP/Qur	273.3 \pm 11.81	253.0 \pm 13.13 ^o	7.4 \downarrow	5.4 \pm 0.3#

Data are expressed as mean \pm SEM, n=8.

^o significantly different from its corresponding initial value using paired Student's t-test (p<0.05).

* significantly different from control group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test (p<0.05).

significantly different from CP-treated group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test (p<0.05).

3.2. Effect of CP and/or Qur on serum total protein, LDH and TNF- α

Administration of CP alone induced a significant increase in serum total protein and LDH activity by 61.4% and 126.6%, respectively compared to control non-treated group (Table 2). Qur-treated group showed non-significant change compared to control non-treated group. Animals treated with CP and Qur showed a significant decrease in serum total protein and LDH by 14.5% and 28.4%, respectively, compared to CP treated group (Table 2).

Table 2. Effect of cyclophosphamide (CP,150 mg/kg) and/or quercetin (Qur, 100 mg/kg) on serum total protein and LDH

Treatment	Total protein (g/dl)	LDH (U/L)
Control	4.898 \pm 0.111	123.7 \pm 4.8
CP	7.903 \pm 0.242*	280.3 \pm 16.3 *
Qur	5.144 \pm 0.183#	115.8 \pm 8.3#
CP/Qur	6.75 \pm 0.29*#	200.7 \pm 4.9*#

Data are expressed as mean \pm SEM, n=8.

*significantly different from control group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test (p<0.05).

#significantly different from CP-treated group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test (p<0.05).

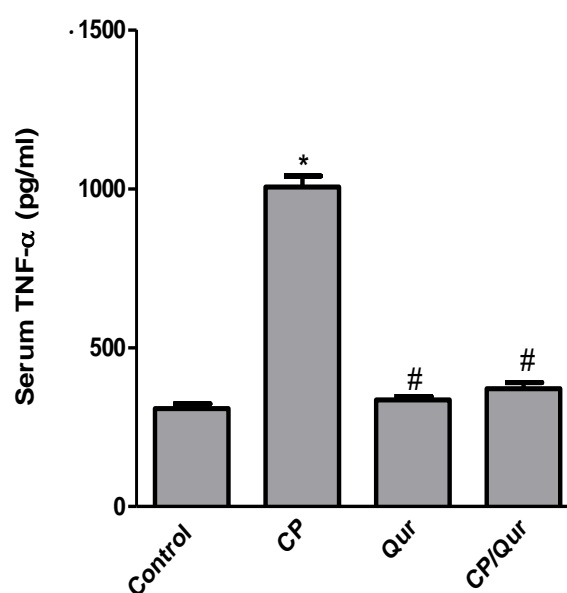


Fig 1. Effect of cyclophosphamide (CP,150 mg/kg) and/or quercetin (Qur, 100 mg/kg) on serum TNF- α .

* significantly different from the corresponding mean value of control group (p<0.05).

significantly different from the corresponding mean value of CP-treated group (p<0.05).

Statistical comparisons were performed using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test (p<0.05).

CP-treated group produced an increase in the level of serum TNF- α by 226.3% compared to control non-treated group. Animals treated with CP and Qur showed a significant decrease in the levels of serum TNF- α compared to CP treated group animals (Figure 1).

3.3. Effect of CP and/or Queron lung content of TBARS, GSH and SOD

CP induced a significant increase in lung TBARS level compared to control non-treated rats by 90%. Administration of Qur for 7 days prior to and 7 days after

CP showed significant decrease in the level of TBARS compared to CP alone by about 29% (Table 3).

CP induced a significant decrease in lung GSH level compared to control non-treated group (83% decrease). Administration of Qur for 7 days prior to and after CP produced a significant increase in lung GSH level compared to CP group (Table 3).

CP-treated group showed a significant increase in lung SOD activity by 95.5%, when compared to control non-treated group. Administration of Qur with CP showed a significant decrease in lung SOD activity when compared to CP group (Table 3).

Table 3. Effect of cyclophosphamide (CP, 150 mg/kg) and/or quercetin (Qur, 100 mg/kg) on lung antioxidant status

Treatment	TBARS (nmol/g wet tissue)	GSH (μ mol/g wet tissue)	SOD (units/g wet tissue)
Control	63.63 \pm 2.322	0.667 \pm 0.0298	27.5 \pm 1.24
CP	121.4 \pm 7.154*	0.112 \pm 0.0093*	53.75 \pm 0.701*
Qur	60.38 \pm 5.003#	0.519 \pm 0.0295#	28.50 \pm 0.98#
CP/Qur	86.0 \pm 6.251#	0.201 \pm 0.021*#	32.50 \pm 2.32#

Data are expressed as mean \pm SEM, n=8.

* significantly different from control group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test ($p < 0.05$).

significantly different from CP-treated group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test ($p < 0.05$).

3.4. Effect of combination of Qur with CP on histopathological analysis in CP-treated rats

Control rats showed no abnormality for the lung histology as well as those treated with Qur alone. In CP-treated animals, histopathological study revealed foci of

oedema and congestion, alveolar septa thickening by macrophages and lymphocytes and the alveolar lining is prominent. Photomicrographs of representative histological sections of the lung can be seen in figure 2.

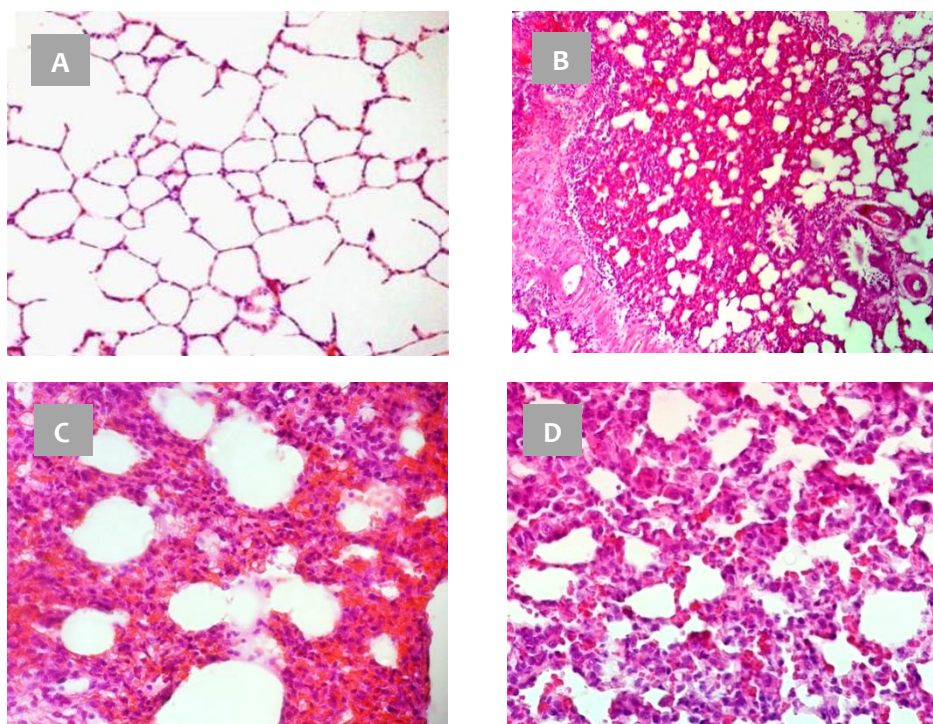


Fig2. Light microscopy photomicrographs of representative histological sections of the lung of:

(A) Control group showing normal appearance (H&E \times 100).

(B) CP group showing foci of oedema and congestion, marked thickening of the alveolar septa and the alveolar lining is prominent (H&E \times 200).

(C) Higher power showing increased cellularity of the alveolar septa by inflammatory cells and extravasated blood (H&E \times 400).

(D) CP/Qur group showing thickening of the alveolar septa in 60% of all examined fields (H&E \times 400).

Lung of the combined group showed thickening of the alveolar septa in 60% of all examined fields.

4. Discussion

The adverse and serious side effects of potent chemotherapeutic agents constitute a major problem that limits their optimal use. CP is one of the most effective chemotherapeutic agents used in treatment of cancers of lung (Zhang et al., 2006), breast (Hirano et al., 2008), ovary (Donato et al., 2004), prostate (Nicolini et al., 2004), leukemia, (Rao et al; 2005) and lymphomas.

Cancer patients usually suffer from lung toxicity after CP therapy (Malik et al., 1996) that is characterized by hypoxemia, non-cardiogenic pulmonary edema, low lung compliance and widespread capillary leakage.

In the present study, the effect of quercetin (Qur) in the treatment of acute lung injury produced from CP was evaluated.

Intraperitoneal injection of CP resulted in significant decrease in body weight, and a significant increase in lung weight index in rats compared to untreated control group. The inflammation and edema after CP treatment may be responsible for the increase in lung weight indices.

Administration of quercetin with CP showed a significant decrease in lung weight index compared to CP alone treated group, this finding is agreement with the study of Carvalho et al.,(2010), in which pretreatment with quercetin significantly attenuated the severity of acute pancreatitis as evidenced by effective reductions in the pancreatic wet weight/body weight ratio, biochemical indices, proinflammatory cytokines as TNF- α , IL-1 β , and IL-6, myeloperoxidase activity, malondialdehyde formation, and an increase in antiinflammatory cytokine IL-10. Quercetin treatment also markedly suppressed the histological changes such as pancreatic edema, inflammatory cell infiltration, acinar cell necrosis, and the expression of TNF- α . Taken together; these results indicate that quercetin acting as an antiinflammatory and antioxidant agent.

In addition, intercellular adhesion molecule-1 (ICAM-1) plays a pivotal role in inflammatory responses, quercetin is an inhibitor of agonist induced ICAM-1 protein and mRNA expression. The down-regulation of this adhesion molecules lead to reduction of inflammation and edema Kobuchi et al., (1999).

In the present study, the production of MDA, which is an index of lipid peroxidation was increased significantly in lung homogenate compared to control group after CP administration. This observation is in line with many reports that demonstrated apparent elevation in lung TBARS following administration of CP (Patel, 1987, Venkatesan and Chandrakasan., 1995, Stankiewicz et al., 2002). Increased MDA level observed following CP administration in the current work could be indirectly attributed to the decreased level of GSH, also CP has been proved to induce significant alterations in the activity of NADH and NADPH₂, likely to be responsible for the ultrastructural changes within mitochondria which include features of mitochondrial

damage with disruption of mitochondrial membranes. We assume that an increase in MDA level in lung homogenates may be a biochemical evidence of these changes.

Administration of quercetin with CP showed a significant decrease in the level of TBARS compared to CP treated rats. This activity may be due to the ability of quercetin to inhibit lipid peroxidation by scavenging oxygen radicals (Jovanovic et al., 1994), terminating the chain-radical reaction, chelating metal ions (Afanas'ev et al., 1989) to form inert complexes that cannot take part in the conversion of superoxide radicals and hydrogen peroxide into hydroxyl radicals.

Also, Şekeroğlu et al., (2011) suggest that quercetin supplementation attenuates CP induced cardiotoxicity, urotoxicity and genotoxicity through a mechanism related to their ability to decrease oxidative stress and inflammation.

Administration of CP in the current study reduced the lung content of GSH by about 83%. This could be due to the decreased expression of this antioxidant during bronchial cellular damage. CP metabolism produces highly reactive electrophiles that lead to electrophilic burden on the cells and also due to the formation of acrolein, which deplete GSH contents (McDiarmid et al., 1991).

This is in line with other reports that demonstrated GSH reduction or depletion following CP challenging in animals.

Patel and Block (1985); Venkatesan and Chandrakasan (1995); Sulkowska et al. (2002); Manda and bhatia (2003) reported that, CP is capable of reducing the level of tissue GSH in both mice and rats. Administration quercetin with CP showed a significant increase in the level of GSH compared to the negative control group, and a significant increase in the level of GSH compared to the CP treated group. These findings are in agreement with the investigation conducted by Sekeroğlu et al. (2010) through which quercetin (50 mg/kg/day) for 10 days alone or in combination with CP showed significant decreases in the level of GSH resulted from this quercetin supplementation attenuates CP induced cardiotoxicity, urotoxicity and genotoxicity through a mechanism related to their ability to decrease oxidative stress, inflammation, its free radicals scavenging activity and its antioxidant activity.

In this study, CP (150 mg/kg) increased the lung activity of SOD by about 95.5% compared to untreated control group. Such controversy could be explained in virtue of the postulate that the CP-induced increase in lung SOD activity is rather a compensatory event that occurred indirectly due to the oxidant injurious effect of CP and not due to a direct effect of the drug

Administration of quercetin with CP showed a significant decrease in the level of lung SOD compared to the positive control group, this inhibition could presumably be explained as a consequence of less availability of the substrates for these enzymes (superoxide radical or free radicals) due to the reported

superoxide radical scavenging effect as SOD is the only known enzyme that uses free radicals as a substrate.

In this study, CP (150 mg/kg) increased serum level of both total protein and LDH compared to negative control group. These events are indicative of airway and/or alveolar cell damage, airway cell influx, and microvascular leakage. In addition, this elevation could potentially be attributed to their release from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage due to generation of reactive oxygen species and other free radicals during CP exposure.

Administration of quercetin with CP showed a significant decrease in serum level of both total protein and LDH compared to positive control group, this could be due to the ability of quercetin to reduce free radical-induced oxidative damage in the lung.

One of the essential mediators of inflammation in human body is the cytokines. TNF- α , one of the chief cytokines, is involved in provoking acute inflammatory responses in high dose CP administration.

In the present study, CP administration showed significant increase in the serum level of TNF- α compared to negative control group.

This result is in agreement with the investigation reported by Kumar et al. (2011) who showed there was a significant rise in the levels of TNF- α in the serum of CP intoxicated rats as compared to that of control rats.

In the present study, administration of quercetin with CP significantly decreased TNF- α level compared to the CP-treated group. This result is in agreement with the investigation reported by Granado-Serrano et al. (2012) at which quercetin attenuated TNF-induced inflammation in hepatic cells by inhibiting the NF- κ B Pathway, also quercetin administration for 4 weeks resulted in decreased expression levels of mRNA for TNF- α and iNOS genes and decreased secretion of TNF- α protein and production of NO by lipopolysaccharides stimulated macrophages from both senescent and young mice (Qureshi et al., 2011).

Histopathological examination of lung specimens isolated from CP-treated rats showed congestion, damage, and/or edema of interalveolar septa, neutrophilic and macrophages infiltration. Sulkowska and sulkowski (1998) demonstrated that congestion and edema may be due to the changes produced by CP in epithelial cell structure as well as alveolocapillary permeability. Treatment with quercetin showed that less lung damage, no areas of intralobular necrosis with less inflammatory infiltration in lung, thickening of the alveolar septa in 60% of examined field.

In conclusion, the results indicate that quercetin partially protects healthy lung cells against cyclophosphamide injury.

5. Acknowledgements

The authors acknowledge Dr. Azza Abdel-Aziz, Department of Pathology, Faculty of Medicine, Mansoura University for providing assistance.

References

- Afanas'ev, I. B.; Dorozhko, A. I.; Brodskii, A. V.; Kontyukm V.A. and Potapovitch, A. I. Chelating and free radical scavenging mechanism of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochemical Pharmacology*, **1989**, 38, 1763-1768.
- Carvalho, K. M.; Morais, T. C.; de Melo, T. S.; de Castro Brito, G. A.; de Andrade, G. M.; Rao, V. S. and Santos, F. A. The natural flavonoid quercetin ameliorates cerulein-induced acute pancreatitis in mice. *Biological and Pharmaceutical Bulletin*, **2010**, 33, 1534-1539.
- Daba, M. H.; Abdel-Aziz, A. H.; Moustafa, A. M.; Al-Majed, A. A.; Al-Shabanah, O. A. and El-Kashef, H. A. Effects of L-carnitine and Ginkgo Biloba extract (EGb 761) in experimental bleomycin-induced lung fibrosis. *Pharmacological Research*, **2002**, 45, 461-467.
- Daniel, W.W. Hypothesis testing. In: *Biostatistics: A Foundation for Analysis in the Health Sciences*: 5th ed. Jhon Wley & Sons. New York, Chichester, Brisbane, Toronto and Singapore, **1991**.
- Donato, M. L.; Aleman, A.; Champlin, R. E. et al. Analysis of 96 patients with advanced ovarian carcinoma treated with high-dose chemotherapy and autologous stem cell transplantation. *Bone Marrow Transplantation*, **2004**, 33, 1219-1224.
- Ellman, G. L. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, **1959**, 82, 70-77.
- Granado-Serrano, A. B.; Martín, M. Á.; Bravo, L.; Goya, L. and Ramos, S. Quercetin Attenuates TNF-Induced Inflammation in Hepatic Cells by Inhibiting the NF- κ B Pathway. *Nutrition and Cancer*, **2012**, 64, 588-598.
- Hirano, A.; Shimizu, T.; Watanabe, O.; Kinoshita, J.; Kimura, K.; Kamimura, M.; Domoto, K.; Aiba, M. and Ogawa, K. Epirubicin and cyclophosphamide followed by docetaxel as primary systemic chemotherapy in locally advanced breast cancer. *Anticancer Research*, **2008**, 28, 4137-4142.
- Jovanovic, S. V.; Steenken, S.; Tomic, M.; Majanovic, A. and Simic, M. G. Flavonoids as antioxidants. *Journal of American Chemical Society*, **1994**, 116, 4846-4851.
- Kanekal, S.; Fraiser L. and Kehrer J. P. Pharmacokinetics, metabolic activation, and lung toxicity of cyclophosphamide in C57/B16 and ICR mice. *Toxicology and Applied Pharmacology*, **1992**, 114, 1-8.
- Kim, S.; Tannock, I.; Sridhar, S.; Seki, J. and Bordeleau, L. Chemotherapy induced infiltrative pneumonitis cases in breast cancer patients. *Journal of Oncology Pharmacy Practice*. **2012**, 18, 311-315.
- Kobuchi, H.; Roy, S.; Sen, C. K.; Nguyen, H. G. and Packer, L. Quercetin inhibits inducible iCAM-1 expression in human endothelial cells through the JNK pathway. *American Journal of Physiology*, **1999**, 277, C403- C411.
- Kumar, S.; Dhankhar, N.; Kar, V.; Shrivastava, M. and Shrivastava, S. Myocardial Injury Provoked by Cyclophosphamide, Protective Aspect of Hesperidin in Rats. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, , 2, 1288-1296.
- Malik, S. W.; Myers, J. L.; DeRemee, R. A. and Specks, U. Lung toxicity associated with cyclophosphamide use. Two distinct patterns. *American Journal of Respiratory and Critical Care Medicine*, **1996**, 154, 2011-1851-1856.

- Manda, K. and Bhatia, A. L. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. *Cell Biology and Toxicology*, **2003**, 19, 367-372.
- Marklund, S. L. Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice. *Mutation Research*, **1985**, 148, 129-134.
- McDiarmid, M. A.; Iype, P. T.; Kolodner, K.; Jacobson-Kram, D. and Strickland, P. T. Evidence for acrolein-modified DNA in peripheral blood leucocytes of cancer patients treated with cyclophosphamide. *Mutation Research*, **1991**, 248; 93-99.
- Nicolini, A.; Mancini, P.; Ferrari, P.; Anselmi, L.; Tartarelli, G.; Bonazzi, V.; Carpi, A. and Giardino, R. Oral low-dose cyclophosphamide in metastatic hormone refractory prostate cancer (MHRPC). *Biomedicine and Pharmacotherapy*, **2004**, 58, 447-450.
- Ohkawa, H.; Ohishi, N. and Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, **1979**; 95, 351-358.
- Patel, J. M. Stimulation of cyclophosphamide-induced pulmonary microsomal lipid peroxidation by oxygen. *Toxicology*, **1987**, 45, 79-91.
- Patel, J. M. and Block, E. R. Cyclophosphamide-induced depression of the antioxidant defense mechanisms of the lung. *Experimental Lung Research*, **1985**, 8, 153-165.
- Pawlikowska-Pawlega, B.; Gruszecki, W. I.; Misiak, L. E. and Gawron, A. The study of the quercetin action on human erythrocyte membranes. *Biochemical Pharmacology*, **2003**, 66, 605-612.
- Qureshi, A. A.; Tan, X.; Reis, J. C.; Badr, M. Z.; Papasian, C. J.; Morrison, D. C. and Qureshi, N. Inhibition of nitric oxide in LPS-stimulated macrophages of young and senescent mice by δ -tocotrienol and quercetin. *Lipids in Health and Disease*, **2011**, 10, 239.
- Rao, R.; Shammo, J. M.; Enschede, S. H.; Porter, C.; Adler, S. S.; Venugopal, P. and Gregory, S. A. The combination of fludarabine, cyclophosphamide, and granulocyte-macrophage colony-stimulating factor in the treatment of patients with relapsed chronic lymphocytic leukemia and low-grade non-Hodgkin's lymphoma. *Clinical Lymphoma*, **2005**, 6, 26-30.
- Ross, J. A. and Kasum, C. M. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annual Review of Nutrition*, **2002**, 22, 19-34.
- Şekeroğlu, V.; Aydın, B. and Şekeroğlu, Z. A. *Viscum album* L. extract and quercetin reduce cyclophosphamide-induced cardiotoxicity, urotoxicity and genotoxicity in mice. *Asian Pacific Journal of Cancer Prevention*, **2011**, 12, 2925-2931.
- Stankiewicz, A.; Skrzydlewska, E. and Makieła, M. Effects of amifostine on liver oxidative stress caused by cyclophosphamide administration to rats. *Drug Metabolism and Drug Interaction*, **2002**, 19, 67-82.
- Sulkowska, M. and Sulkowski, S. Alveolar cells in cyclophosphamide-induced lung injury. An ultrastructural analysis of type II alveolar epithelial cells in situ. *Histology and Histopathology*, **1998**, 13, 13-20.
- Sulkowska, M.; Skrzydlewska, E.; Sobaniec-Łotowska, M. et al. Effect of cyclophosphamide-induced generation reactive oxygen forms on ultrastructure of the liver and lung. *Bull Vet Inst Pulawy*, **2002**, 46; 239-246.
- Venkatesan, N. and Chandrakasan, G. Modulation of cyclophosphamide-induced early lung injury by curcumin, an anti-inflammatory antioxidant. *Molecular and Cellular Biochemistry*, **1995**, 142, 79-87.
- Vouldoukis, I.; Lacan D.; Kamate C.; Coste P.; Calenda A.; Mazier D.; Conti M. and Dugas B. Antioxidant and anti-inflammatory properties of a Cucumis melo LC. extract rich in superoxide dismutase activity. *Journal of Ethnopharmacology*, **2004**, 94, 67-75.
- Zhang, J.; Tian, Q. and Zhou, S. Clinical Pharmacology of Cyclophosphamide and Ifosfamide. *Current Drug Therapy*, **2006**, 1, 55-84.