

Pulmonary Responses Following Quercetin Administration in Rats After Intratracheal Instillation of Amiodarone

Oka V. O.*, Okon U. E. and Osim E. E.

Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Cross River, Nigeria

Summary: Amiodarone, a drug that treats arrhythmias induces pulmonary toxicity through interplay between oxidative stress and inflammation. Quercetin, a flavonoid widely occurring in natural products possesses antioxidant and anti-inflammatory properties. The aim of the present study was to evaluate the effects of quercetin on pulmonary responses in rats after amiodarone intra-tracheal instillation. Eighteen female Wistar rats (150-250 g) were randomly assigned into three groups of six animals each namely; control, amiodarone (AMI) and amiodarone + Quercetin (AMI + Quercetin) groups. AMI group received 2 intra-tracheal instillations of amiodarone (6.25mg/kg in 0.3ml of water) on days 0 and 2 and 0.4ml of 2% DMSO (Dimethyl sulfoxide) orally from day 0 for 3 weeks. AMI + Quercetin group was administered 2 intratracheal instillations of amiodarone on days 0 and 2 and 20mg/kg body weight of quercetin in 2% DMSO from day 0 for 3 weeks. Thereafter, the animals were sacrificed and bronchoalveolar lavage fluid (BALF) was collected to determine total cell polymorphonuclear (PMN) cell and macrophage counts. Inflammation of the lung tissues was also assessed. Macrophage count of AMI + Quercetin group was significantly lowered ($p < 0.01$) compared to AMI group. Inflammation rate of the AMI + Quercetin group was significantly reduced compared to AMI group ($p < 0.01$). Quercetin treatment markedly suppressed amiodarone induced toxicity in the pulmonary tissues.

Keywords: Amiodarone, Quercetin, Pulmonary inflammation, Bronchoalveolar lavage fluid, Intra-tracheal instillation
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*Address for correspondence: vicoka83@gmail.com

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INTRODUCTION

Amiodarone, an iodine-containing compound is a potent anti-arrhythmic agent widely used in the treatment of supra-ventricular and ventricular arrhythmias (Nacca et al., 2012) with a possibility of playing a role in post-myocardial infarction mortality reduction (Chen and Hedges, 2003). It has numerous well-described biochemical and electrophysiological effects (Vasallo and Trohman, 2007; Zimetbaum, 2007). Amiodarone has prophylactically been used in the peri-operative period in patients undergoing thoracic surgery including coronary revascularization and valve replacement (Aasbo et al., 2005).

Despite its vital role in relieving cardiac related problems, it is however associated with variety of adverse effects involving many different organs and system (Wolkove and Baltzan, 2009; Al-shammari et al., 2016). It tends to accumulate in several organs, including the lungs (Wolkove and Baltzan, 2009). Of all the adverse effects of amiodarone, the most serious, feared and potentially life threatening is Amiodarone-induced Pulmonary toxicity (APT) (Nacca et al., 2012; Wolkove and Baltzan, 2009; Al-shammari et al., 2016).

Amiodarone and its metabolites produce lung damage directly by a cytotoxic effect and also indirectly by an immunological reaction (Martin and Rosenow, 1988). The active principal metabolite, desethylamiodarone, penetrates tissues and accumulates therein, thus providing a sustained source of release (Nacca et al., 2012). Direct damage to cells occurs through the production of toxic O₂ radicals (Wolkove and Baltzan, 2009). The indirect effect is supported by the report of the presence of cytotoxic T cells in BALF from patients diagnosed with APT (Jessurum and Criijns, 1997). APT is partly characterized as oedema, phospholipidosis, inflammation and thickening of the alveolar septa, intra-alveolar inflammation, as well as lung infection (pneumonia), as well as pulmonary fibrosis (Mason, 1987; Martin and Rosenow, 1988). Other, more localized forms of pulmonary toxicity may occur, including pleural disease, migratory infiltrates and single or multiple nodules (Wolkove and Baltzan, 2009).

Intra-tracheal instillation is a technique of introducing substances directly into the trachea. It is widely used in assessing respiratory toxicity of a substance as an alternative to inhalation in animal

testing (Driscoll et al., 2005). In the present study, intra-tracheal administration of amiodarone was employed to induce pulmonary toxicity.

Quercetin, a bioflavonoid widely distributed in nature and found in many grains, leaves, fruits and vegetables (Shaik et al., 2006) is used as ingredient in supplements or foods (Formica and Regelson, 1995). Studies have reported that quercetin inhibits the oxidation of other molecules and therefore serves as an anti-oxidant (Williams et al., 2004; Russo et al., 2014). It contains polyphenolic chemical structure which halts oxidation of free radicals that mediate oxidative chain reactions (Murakami et al., 2008). Also, quercetin is reported to possess anti-inflammatory properties (Shaik et al., 2006). It inhibits the growth of certain malignant cells in vitro, and histamine and most cyclin-dependent kinases and also displays unique anti-cancer properties (Shaik et al., 2006; Russo et al., 2014). A study reported that quercetin is a non-specific protein kinase enzyme inhibitor (Russo et al., 2014) that activates or inhibits the activities of a number of proteins (Feitelson et al., 2015) and blocks substances involved in allergies by inhibiting mast cell secretion.

In spite of enormous information as regards the induction of pulmonary fibrosis by amiodarone, the management of this lung disorder is frequently difficult and unfortunately, therapeutic approaches to inhibit the development of amiodarone pulmonary toxicity are scanty (Punithavathi et al., 2003). As a natural therapy with numerous beneficial effects including anti-inflammatory and anti-oxidant effect, it is conceivable that quercetin can influence pulmonary toxicity. Hence, the present study was carried out to evaluate effects of quercetin on pulmonary responses in the lungs of rats after intra-tracheal administration of Amiodarone.

MATERIALS AND METHODS

Chemicals: Amiodarone, ketamine hydrochloride, dimethyl sulfoxide (DMSO) and quercetin were purchased from Medreich plc, UK, Kwaliti Pharmaceuticals Pvt, India, Guanghua Chemical, China and Sigma, USA respectively.

Animals: Eighteen female Wistar rats weighing between 150-250 g were used for this study. They were procured from the Animal house of the Department of Physiology, College of Medical Sciences, University of Calabar, Nigeria. The animals were kept in well ventilated cages at room temperature 25±2°C and exposed to a normal 12/12 hours light/dark cycle. They had access to rodent chow and clean drinking water ad libitum. All procedures involving animal handling followed the National Institutes of Health guide for the care and use of Laboratory animals (NIH publications No. 8023, revised 1978) and approved by the Faculty of Basic Medical Sciences Animal Ethics Committee.

Experimental Protocol: The animals were weight matched into three groups: Control (CTL), Amiodarone (AMI) and Amiodarone plus quercetin (AMI + Quercetin) groups. The CTL group received 0.3 ml of distilled water (vehicle) by intra-tracheal instillation on days 0 and 2 and 0.4 ml of 2% DMSO orally from day 0 for 3 weeks. The AMI group received 2 intra-tracheal instillations of amiodarone (6.25 mg/kg in 0.3 ml of water) on days 0 and 2 and 0.4 ml of 2% DMSO orally from day 0 for 3 weeks. The AMI + Q group was treated with 2 intra-tracheal instillations of amiodarone on days 0 and 2 and 20mg/kg body weight of quercetin in 2% DMSO from day 0 for 3 weeks. Drugs and vehicle dosages were based on previous studies (Hamid-Reza et al., 2016; Ujah et al., 2016).

At the end of the study period, the animals were sacrificed under deep anaesthesia by ingestion of pentobarbital and dissected to remove the lungs. BALF was collected by inserting a cannula into the right lung via the respiratory tract, with the left main bronchus clamped, and pouring in a physiological saline (5 to 10 mL each). Fifty ml of BALF per group was centrifuged (1500 rpm; 10 min) to separate the cellular components. After 1 mL of buffer was added and stirred, the fluid was examined for macrophages and neutrophils (cell/μL) with an automatic blood cell counter (Celltac MEK 5204 Nihon Koden, Tokyo). The results were expressed as the total number of cells in the BALF.

Also, from the samples of the BALF collected, smears were made and stained. The slides were then observed by an optical microscope at ×400 magnification; the alveolar macrophages (AM) and PMN in the BALF were identified by their shape. The number of PMN in the BALF was obtained by multiplying the percentage of PMN by the total number of cells (Okada et al., 2016).

Histopathology of the Lung: The left lung of each rat (the clamped side in the BALF collection) was fixed with 10% buffered formalin. Paraffin sections of the left lung (3 μm thickness) were stained with hematoxylin eosin (HE).

Point count method for HE staining: This method was adapted by Okada et al., (2016). Lung tissue specimens are stained. After staining, digital images of each lung section were photographed under light microscopy at x100 magnification. In order to eliminate measurer bias, a third person randomly assigned numbers from 1 to 90 to microscope photographs per time point and returned them to the measurer. The images were chiefly focused on the alveoli. Photographs obtained were returned to the original order through the use of conversion table. Place over each image on a computer screen was a 300-point grid. Pulmonary inflammation at each time point was examined using the point counting method (PCM) (Ogami et al., 2004). The

accumulation of macrophages and PMN was counted mainly as inflammatory change. The rate of points of inflammation was calculated as shown; ($I=X/300$)

I = inflammation rate,
X = the number of inflammation points among 300 points.

Statistical Analyses: All data were analyzed by one-way analysis of variance followed by post hoc student Tukey test using Graph pad prism version 7.01. Values are presented as mean \pm S.E.M. Significance was accepted at $P < 0.05$.

RESULTS

The total cell count of both the AMI and AMI + Quercetin groups was not significantly different compared to the control. However, the value for the AMI + Quercetin group was significantly lower ($p < 0.01$) compared to AMI group (Figure 1). The polymorphonuclear cell count of both the AMI and AMI +

Quercetin groups showed no significant difference compared to the control (Figure 2).

Figure 3 showed that the macrophage count in the AMI group was significantly higher ($p < 0.001$) compared to control. The value for AMI + Quercetin group was significantly lower ($p < 0.01$) compared to AMI group. The HE-stained images of the lung tissues following quercetin administration after amiodarone intra-tracheal instillation are shown in Figure 4. In Figure 4A, the lung tissue section shows prominent alveoli spaces with intact linings. The intervening inter-alveoli septa are thin. Numerous collapsed alveoli spaces and widened inter-alveoli septa consisting of mononuclear inflammatory cells and proliferative pneumocytes were observed in the AMI group (Figure 4B). There is marked inflammation around the terminal bronchioles. In the AMI + Quercetin group, the inter-alveoli septa were observed to be mildly thickened. Also a few of the alveoli spaces are dilated with intact epithelial cells (Figure 4C).

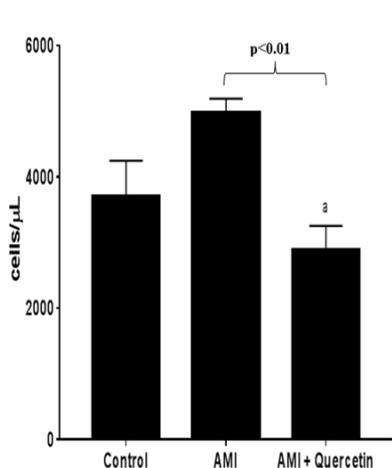


Fig.1: Total cell count in control, AMI and AMI + Quercetin experimental groups. Values are expressed as Mean \pm SEM, n=6, a= $p < 0.01$ vs AMI

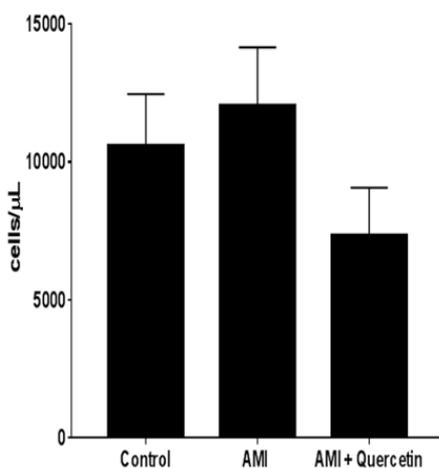


Fig.2: Polymorphonuclear cell count in control, AMI and AMI+ Quercetin experimental groups. Values are expressed as Mean + SEM, n=6

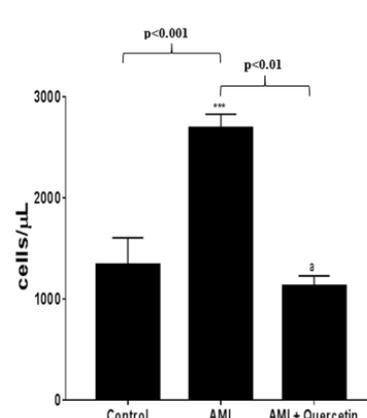


Fig. 3: Macrophage count in control, AMI and AMI + Quercetin experimental groups. Values are expressed as Mean \pm SEM, n= 6. ***= $p < 0.001$ vs control, a= $p < 0.01$ vs AMI

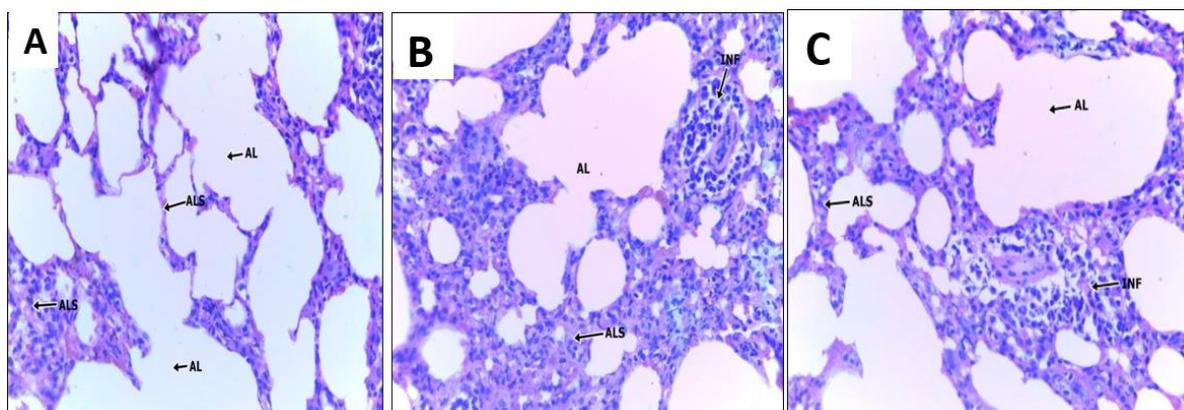


Fig.4: Lung sections(x400) with HE stain at 3 days for the (A) Control: section of lung tissues show prominent alveoli spaces (AL) with intact linings consisting of prominent pneumocytes. The alveoli spaces are dilated and empty of secretion and the intervening inter-alveoli septa (ALS) are thin. (B) AMI: section of lung tissues shows numerous collapsed alveoli spaces (AS) and widen inter-alveoli septa (ALS) consisting of mononuclear inflammatory cells and proliferating pneumocytes. There is marked inflammation around the terminal bronchioles bronchioles. Few of the alveoli spaces are dilated, lined by flattened intact epithelial cells and empty (C) AMI + Quercetin groups: section of lung tissues shows numerous collapsed alveoli spaces separated by mildly thickened inter-alveoli septa consisting of mild mononuclear inflammatory cells and proliferating pneumocytes. Few of the alveoli spaces are dilated, lined by flattened intact epithelial cells and empty.

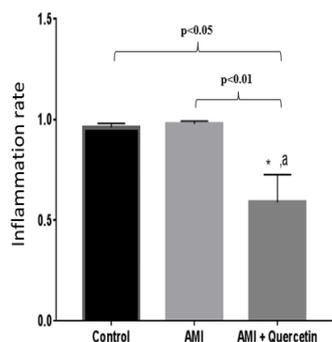


Fig.5: Inflammation rate in control, AMI and AMI + Quercetin experimental groups. Values are expressed in Mean ± SEM, n=6. *= $p<0.05$ vs control, a= $p<0.01$ vs AMI.

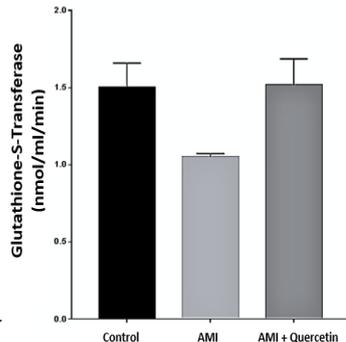


Fig. 6: Glutathione-S-Transferase levels in control, AMI and AMI + Quercetin experimental groups. Values are expressed in Mean ± SEM, n=6.

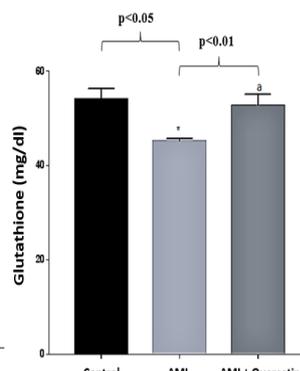


Fig. 7: Glutathione levels in control, AMI and AMI + Quercetin experimental groups. Values are expressed as Mean ± SEM, n=6. *= $p<0.05$ vs control, a= $p<0.01$ vs AMI.

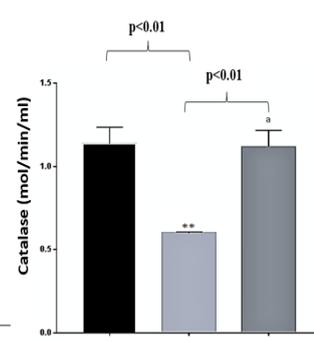


Fig. 8: Catalase levels in control, AMI and AMI + Quercetin experimental groups. Values are expressed in Mean ± SEM, n=6. **= $p<0.01$ vs control, a= $p<0.01$ vs AMI.

The inflammation rate of the AMI group was not significantly different compared to control, whereas the value for AMI + Quercetin group was significantly lower compared to both control and AMI groups ($p<0.05$ and $p<0.001$ respectively) (Figure 5).

The Glutathione-S-Transferase levels of both the AMI and AMI + Quercetin groups were not significantly different from the control, although that of AMI + Quercetin group appeared to be slightly higher compared to the AMI group (Figure 6).

While the Glutathione level of the mice in the AMI group was significantly lower compared to control ($p<0.05$), the value for AMI + Quercetin group was observed to be significantly increased compared to the AMI group ($p<0.01$) (Figure 7).

The Catalase level of AMI group was observed to be significantly lower compared to the control ($p<0.01$), whereas that of AMI + Quercetin group was higher compared to AMI group with a significance of $p<0.01$ (Figure 8).

DISCUSSION

In the present study, total cell count was observed to be higher in amiodarone administered rats. This is also in line with the report of Punithavathi et al. (2003). This increase might have been as a result of the reaction of the body to the drug, a response to fight off any infection. Quercetin treatment resulted in marked reduction in total cell count. This report is consistent with that of Liu et al., (2013), who reported a reduction in total cell count following quercetin administration.

PMN, the first line of defence against invading pathogens was observed to have a slightly increased count following amiodarone intra-tracheal instillation. Quercetin was observed to lower the PMN cell count of the animals. Quercetin might have lowered the PMN by inhibiting several factors activated under inflammatory conditions (Gonzalez et al., 2011). This is consistent with the report of Nikfarjam et al. (2017), who stated a reduction of PMN, occurred as a result of

the reduction of nitric oxide (NO), tumour necrosis factor α (TNF- α) and myeloperoxidase (MPO).

Macrophage count of the amiodarone only treated group was significantly higher than the control. Our data is consistent with that of the study conducted by El-Mohandes et al., (2017). These macrophages appear to play vital roles in amiodarone induced pulmonary fibrosis (El-Mohandes et al. 2017). This cellular response is indicative of the development of a drug-induced phospholipidosis (Reasor et al., 1988).

The HE stained images of the lung tissues of the AMI group showed many collapsed alveoli spaces and widened inter-alveoli septa. Also, there was marked pneumonitis and vacuolization of the bronchial epithelial cell. This is supported by the report of Wolkove and Baltzan (2009). This hyperplasia of the pneumocytes and alveolar septa widening may be as a result of the toxic O_2 radical production, which damage the cells directly and also promote the accumulation of phospholipids in tissues (Jessurum and Crijns, 1997; Halliwell et al., 1997). This amiodarone-induced phospholipidosis triggered the formation of lip-laden macrophages as observed in the present study.

Administration of quercetin reduced thickening of the inter-alveoli septa. The antioxidant activity of quercetin as observed in the present study may be attributed to many factors including free radical scavenging, protection against lipid oxidation or up-regulation of antioxidant enzymes (Liu et al., 2013). Heijnen et al. (2002), reported that structural features allowed quercetin to donate hydrogen to scavenge free radicals and increase the stability of flavonoid radicals.

Inflammation, the most common appearance of tissue pathology, is involved in the pathogenesis of many disease conditions including APT. The data obtained from our study showed suppression of the inflammatory response (triggered by amiodarone instillation) of the lung tissues following quercetin administration. This result is consistent with the report of Nikfarjam et al. (2017). Quercetin has been reported to suppress inflammation by reducing TNF- α and NO synthase production in obese Zucker rats.

The anti-inflammatory effects of quercetin as observed in the present study may be attributed to the interaction between oxidative stress and inflammation (Boots et al., 2008). Reactive oxygen species (ROS) scavenging by quercetin not only prevents oxidative stress, but also mitigates inflammation. This is because ROS are not only involved in the occurrence of oxidative stress but likewise in the promotion of inflammatory processes (Madamanchi et al., 2005). They also induce production of TNF- α , which also trigger inflammation (Rahman, 2002).

Study by Nair et al. (2006) reported that quercetin inhibited TNF- α production and gene expression through nuclear factor (NF)- κ B modulation. Hence, it is likely that suppressed inflammatory responses observed in this study may be due to inhibition of TNF- α production by quercetin.

The significant increase of the levels of superoxide dismutases including catalase and glutathione following quercetin administration further highlights or serves as pointers to the cytoprotective tendencies of quercetin. This is because these enzymes help in protecting cells from oxidative damage by reactive oxygen species (ROS). This is supported by Pritchard et al., (2014) and Kerksick and Willoughby (2005). In conclusion, we suggest that, intra-tracheal instillation of amiodarone triggered a cascade of immunological responses in the lung tissues of rats. These responses which are deleterious and indicative of pulmonary damage, were ameliorated following quercetin administration.

REFERENCES

Aasbo, J.D., Lawrence, A.T., Krishnan, K., Kim, M.H., Trohman, R.G. (2005). Amiodarone prophylaxis reduces major cardiovascular morbidity and length of stay after cardiac surgery: A Meta-analysis. *Annals of Internal Medicine*, 143: 327-336.

Al-Shammari, B., Khalifa, M., Bakheet, S.A., Yasser, M. (2016). A mechanistic study on the amiodarone-induced pulmonary toxicity. *Oxidative Medicine and Cellular Longevity*. 2016: Article ID: 6265853. doi:10.1155/2016/6265853.

Boots, A.W., Haenen, G.R., Bast, A. (2008). Health effects of quercetin: from antioxidant to nutraceutical. *European Journal of Pharmacology*. 585: 325-337.

Chen, D., and Hedges, T.R. (2003). Amiodarone optic neuropathy-review. *Seminars in Ophthalmology*. 18: 169- 173.

Driscoll, K.E., Costa, D.L., Hatch, G., Henderson R., Oberdorster G., Salem H., Schlesinger R. B. (2000). Intra-tracheal instillation as an exposure technique for the evaluation of respiratory tract toxicity: uses and limitations. *Toxicological Sciences*. 55: 24-35.

El-Mohandes, E.M., Moustafa, A.M., Khalaf, H.A., Hassan, Y.F. (2017). The role of mast cells and fibrosis and the possible attenuating role of ator

vastatin. *Biotechnology and Histochemistry* 92: 467- 480.

Feitelson, M.A., Arzumanyan, A., Kulathinal, R.J, Blain S.W., Holcombe R.F., Mahajna J., Martinez-Chantar M.L., Nawroth R., Sanchez-Garcia I., Sharma D., Saxena N.K., Singh N., Vlachostergios P.J., Guo S., Honoki K., Fujii H., Georgakilas A.G., Bilsland A., Amedei A., Niccolai E., Amin A., Ashraf S.S., Boosani C.S., Guha G., Ciriolo M.R., Aquilano K., Chen S., Mohammed S.I., Azmi A.S., Bhakta D., Halicka D., Keith W.N., Newsheen S. (2015). Sustained proliferation in cancer: mechanisms and novel therapeutic targets. *Seminars in Cancer Biology*. 35 Suppl: S25-S54.

Formica, J.V., and Regelson, W. (1995). Review of the biology of quercetin and related bioflavonoids. *Food and Chemical Toxicology*. 33: 1061-1080.

González, R., Ballester, I., Lopez-Posadas, R., Suarez M.D., Zarzuelo A., Martinez-Augustin O., Sanchez de Medina F. (2011). Effects of flavonoids and other polyphenols on inflammation. *Critical Review of Food Science and Nutrition*. 51: 331-362.

Halliwell, W. (1997). Cationic amphiphilic drug-induced phospholipidosis. *Toxicology and Pathology*. 25: 53-60.

Hamid-Reza, N., Siyavash, J., Hamid, K., Hamid-Reza, P., Shahriar, D. (2016). Coadministration of Atorvastatin and Amiodarone Increases the Risk of Pulmonary Fibrosis in Rats. *Medical Principles and Practice*. 25: 150 – 154.

Jessurum, G.A., and Crijins, H.J. (1997). Amiodarone pulmonary toxicity. *British Medical Journal*. 314: 619-620.

Kerksick, C., Willoughby, D. (2005). The antioxidant role of Glutathione and N-Acetyl-Cysteine supplements and exercise-induced oxidative stress. *Journal of the International Society of Sports Nutrition*. 2: 38-44.

Liu, H., Xue, J., Xing, L., Ao, R., Lu, Y. (2013). Quercetin liposomes protect against radiation-induced pulmonary injury in a murine model. *Oncology Letters*. 6: 453-459.

Madamanchi, N.R., Hakim, Z.K., Runge, M.S. (2005). Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. *Journal of Thrombosis and Haemostasis*. 3: 254-267.

Martin, W.J., Rosenow, E.C. (1998). Amiodarone pulmonary toxicity. Recognition and pathogenesis (Part 2). *Chest*. 93: 1242-1246.

Mason, J.W. (1987). Amiodarone. *The New England Journal of Medicine*. 316: 455-466.

Murakami, A., Ashida, H., Terao, J. (2008). Multi-targeted cancer prevention by quercetin. *Cancer Letters*. 269: 315-325.

Nacca, N., Bhamidipati, C.M., Yuhico, L.S., Pinnamaneni, S., Szombathy, T. (2012). Severe

- amiodarone induced pulmonary toxicity. *Journal of Thoracic Diseases* 4: 667- 670.
- Nair, M.P., Mahajan, S., Reynolds, J.L., Aalinkeel, R., Nair, H., Schwartz, S.A., Kandaswami C. (2006). The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-Kappa beta system. *Clinical and Vaccine Immunology* 13: 319-328.
- Nikfarjam, B.A., Hajiali, F., Adineh, M., Nassiri-Asl, M. (2017). Anti-inflammatory effects of Quercetin and Vitexin on activated human peripheral blood neutrophils. *Journal of Pharmacopuncture*. 20: 127-131.
- Ogami, A., Morimoto, Y., Yamato, H., Oyabu, T., Kajiwara, T., Tanaka, I. (2004). Patterns of histopathological change determined by the point counting method and its application for the hazard assessment of respirable dust. *Inhalation Toxicology*. 16: 793-800.
- Okada, T., Ogami, A., Lee, B.W., Kadoya, C., Oyabu, T., Myojo, T. (2016). Pulmonary responses in rat lungs after intratracheal instillation of 4 crystal forms of titanium dioxide nanoparticles. *Journal of Occupational Health*. 58: 602-611.
- Oostveen, E.M., Stalpers, Bast, A. (2002). Protection of flavonoids against lipid peroxidation: the structure activity relationship revisited. *Free Radical Research*. 36: 575-581.
- Pritchard, R.E., Prassinis, A.J., Osborne, J.D., Raviv, Z., Balish, M.F. (2014). Reduction of Hydrogen Peroxide accumulation and toxicity by a catalase from *Mycoplasma iowae*. *PLoS ONE*. 9: e105188.
- Punithavathi, D., Venkatesan, N., Babu, M. (2003). Protective effects of Curcumin against amiodarone-induced pulmonary fibrosis in rats. *British Journal of Pharmacology*. 139: 1342-1350.
- Rahma, I. (2002). Oxidative stress, transcription factors and chromatin remodelling in lung inflammation. *Biochemical Pharmacology*. 64: 935-942.
- Reasor, M.J., Ogle, C.L., Walker, E.R., Kacew, S. (1988). Amiodarone-induced phospholipidosis in rat alveolar macrophages. *American Review of Respiratory Disease*. 137: 510-518.
- Russo, G.L., Russo, M., Spagnuolo, C., Tedesco, I., Bilotto S., Iannitti, R., Palumbo, R. (2014). Quercetin: a pleiotic kinase inhibitor against cancer. *Cancer Treatment and Research*. 159: 185-205.
- Shaik, Y.B., Castellani, M.L., Perrella, A., Conti, F., Salini, V., Tete, S., Madhappan, B., Vecchiet, J., De Lutiis, M.A., Caraffa A., Cerulli G. (2006). Role of quercetin (a natural herbal compound) in allergy and inflammation. *Journal of Biological Regulators and Homeostatic Agents*. 20: 47-52.
- Ujah, G.A., Nna, V.U., Agah, M.I., Omue, L.O., Leku, C.B., Osim, E.E. (2018). Effect of Quercetin on Cadmium Chloride induced impairments in sexual behaviour and steroidogenesis in male Wistar rats. *Andrologia*. 50. doi:10.1111/and.12866.
- Vasallo, P., Trohman, R.G. (2007). Prescribing amiodarone. *JAMA*. 298: 1312-1322.
- Williams, R.J., Spencer, J.P., Rice-Evans, C. (2004). Flavonoids: anti-oxidants or signalling molecules. *Free Radical Biology & Medicine*. 36: 838-849.
- Wolkove, N., and Baltzan, M. (2009). Amiodarone pulmonary toxicity. *Canadian Respiratory Journal* 16: 43-48.
- Zimetbaum, P. (2007). Amiodarone for atrial fibrillation. *New England Journal of Medicine*. 356: 935-941.