Antioxidant activity and free radical scavenging potential of alpha lipoic acid and quercetin against Al_2O_3 nanoparticle-induced toxicity in mice

Rupal Shrivastava, Rakesh Bhargava, S.J.S. Flora

Division of Regulatory Toxicology, Defence Research and Development Establishment, Jhansi Road, Gwalior 474 002, India

Submission Date: 14-11-2013

ABSTRACT

Introduction: Exposure to nanomaterials (NPs) may lead to enhanced generation of free radicals and failure of endogenous antioxidant defense system ultimately resulting in oxidative stress. The present study aimed to evaluate the comparative efficacy of alpha lipoic acid (ALA) and quercetin, the two antioxidants against aluminium oxide nanoparticle induced oxidative stress. **Methods and Materials:** Male swiss albino mice were exposed to aluminum oxide NPs for seven days and were co-administered orally with quercetin and alpha lipoic acid (50 mg/kg each) to evaluate effects on heme synthesis pathway, hepatic oxidative injury and antioxidant potential. **Results:** The results suggest a significantly elevated ROS, decreased blood and hepatic GSH levels, Superoxide dismutase and Catalase activities after Al_2O_3 nanoparticles exposure. Co-administration of antioxidants increased GSH levels and was also beneficial in the recovery of oxidative injury and restoring inhibited ALAD activity. **Conclusion:** Our results suggest better efficacy of alpha lipoic acid than Quercetin in preventing appearance of toxic symptoms of following exposure to Al_2O_3 nanoparticles in mice.

Keywords: Al_2O_3 nanoparticle; alpha lipoic acid; Quercetin; Protection; Free radicals; Lipid peroxidation.

INTRODUCTION

Nanotechnology is an actively growing field with rapid advances in numerous applications such as in pharmaceutical industries, personal care products, targeted drug delivery etc.[1] Aluminum oxide (Al_2O_3) nanomaterials has promising technological applications in the field of site-specific drug delivery, explosives lithium batteries, resistant coatings on propeller shafts, car finishing and flooring, and orthopedic implants etc[2]. However indiscriminate use of nano aluminium may lead to release of the oxidized form of nano Al_2O_3 into the environment and may produce adverse effects, such as genetic damage, carcinogenicity, cytotoxicity etc.[1] Their small size facilitate cellular uptake and transcytosis across epithelial and endothelial cells reaching into systemic circulation and affecting potentially sensitive target sites such as brain, bone marrow, lymph nodes, spleen, and heart.[3] Nanoparticles can also gain access to the CNS, thus resulting in severe neurotoxic manifestations also.[4] The commonly proposed pathogenic mechanisms initiated by NPs are generation of oxidative stress thereby triggering injurious responses at the cellular, subcellular, and protein levels.[5] Overall, these facts necessitate the study of environmental impact and toxicity of nanoparticles and also highlight the importance for the development of effective preventive and therapeutic strategies.

Flavonoids are a group of natural products, including various classes such as flavones, flavanones, and isoflavones. Many beneficial and therapeutic biological activities of flavonoids have been identified such as antioxidant, antitumor, and anti-inflammation properties.[6] Presence of hydroxyl (–OH) groups and B-ring catechol group (dihydroxylatedB-ring),, serves as a critical factor in the scavenging of reactive oxygen species (ROS) exhibiting potential antioxidant activity.[7] However the antioxidant activity of these flavonoids largely depends upon the

***Corresponding address:**
Dr. S.J.S. Flora, Defence Research and Development Establishment, Jhansi road, Gwalior (M.P.).
E-mail: sjsflora@hotmail.com;
sjsflora@drde.drdo.in

DOI: 10.5530/fra.2014.1.3

presence of functional groups capable of binding transition metal ions, such as iron and copper.[8, 9] Quercetin was found to be the most active of all the flavonoids and has demonstrated significant ability to scavenge free radicals and reduce the oxidative stress conditions.[10,11] Lipoic acid is another potent antioxidant that can scavenge free radicals an thus implicated in the treatment of a variety of diseases.[6] It exerts their protective function in both oxidized as well as in reduced form. Reduced form of alpha-lipoic acid (dihydroxy lipoic acid (DHLA)) acts as a strong chain breaking antioxidant and may enhance the antioxidant potency of other antioxidants.[12] Thus some common and desired properties of alpha-lipoic acid and quercetin such as free radical scavenging activity and structural presence of thiol moiety justify its use in the present study against nanoparticle toxicity. We have demonstrated the antioxidant potential of quercetin and lipoic acid against metal/metalloid- induced oxidative stress in mice.[13–15] The present study was aimed at investigating the comparative protective efficacy of alpha- lipoic acid and quercetin against nanoparticle induced oxidative injury. Liver was selected as the major organ for investigation as it is the major target for free radical attack leading to lipid peroxidation.[6]

MATERIALS AND METHODS

Chemicals and reagents

Alumina (Al_2O_3) nanoparticles were procured from Alfa Aesar (USA). All other laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or BDH chemicals (Mumbai, India). Triple distilled water prepared by Millipore (New Delhi, India) was used throughout the experiment. According to the information provided by manufacturer the particles size of Al_2O_3 nanoparticles was 45 nm. Prior to dosing the suspension was sonicated for 30 minutes.

Animals and treatment

Swiss albino male mice weighing approximately 25–30 g were obtained from the animal house facility of the Defence Research and Development Establishment (DRDE), Gwalior. All animals received humane care in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Animal Ethical Committee of DRDE, Gwalior, India approved the protocols for the experiments. Prior to dosing, they were acclimatized for 7 days to light from 06:00 to 18:00 h, alternating with 12 h darkness. The animals were housed in stainless steel cages in an air-conditioned room with temperature maintained at 25 ± 2°C. Mice were allowed standard chow diet (Amrut feeds, Pranav Agro, New Delhi, India) throughout the experiment and water *ad libitum*.

Twenty four mice were randomized into six groups of 4 animals each and were treated as follows for 7 days:

Group I:	Normal Animal (received normal water)
Group II:	Al_2O_3 nanoparticles (100 mg/kg b.w.) oral, once, daily
Group III:	Quercetin (50 mg/kg b.w.) oral, once, daily
Group IV:	Alpha-lipoic acid (50 mg/ kg b.w.) oral, once, daily
Group V:	Quercetin (50 mg/kg b.w) + Al_2O_3 NPs (100 mg/kg b.w.)
Group VI:	Alpha lipoic acid (50 mg/ kg b.w) + Al_2O_3 NPs (100 mg/kg b.w.)

Antioxidants were administered after an interval of 4 hrs from the oral dosage of nanoparticles. Doses were selected on the basis of earlier publications.[16–18] Oral route of exposure was selected for mice as these nanoparticles are generally being used in various products like food packaging, cosmetics and coating etc and there is possibility of gaining a direct entry into the body. After 7 days the exposure was stopped and animals were sacrificed under light ether anesthesia, 48 h, after last dosing. Blood was collected in heparinized vials. Liver was collected and stored at −80°C till further analysis. For biochemical estimation, the tissues were washed with cold normal saline, blotted and all the extraneous materials were removed.

Separation of red blood cells

Blood was collected from orbital plexus of mice in heparinized tubes following the method of Steck and Kant.[19] The packed cell volume (PCV) obtained was divided into two part, one part of which was diluted with chilled distilled water kept for the analysis of reactive oxygen species (ROS), catalase, thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH). Other part of the packed cell volume was used for the estimation of other antioxidant enzyme i.e. superoxide dismutase (SOD).

Biochemical assays

Amount of ROS in blood was measured using 2', 7'-dichlorofluorescein diacetate (DCF-DA) that gets converted

into highly fluorescent DCF by cellular peroxides (including hydrogen peroxide). The assay was performed as described by Socci et al.[20] Fluorescence was determined at 488 nm excitation and 525 nm emission using a fluorescence plate reader (Tecan Spectra Fluor Plus).

The activity of blood δ-aminolevulinic acid dehydratase (ALAD) was assayed according to method of Berlin and Schaller[21] while, analysis of blood GSH concentration was performed by the method described by Jollow et al.[22] Superoxide dismutase (SOD) activity in liver and blood was assayed spectrophotometrically as described by Kakkar et al.[23] Color intensity of the chromogen was measured at 560 nm and the activity was expressed as units/min/mg of protein. Catalase activity in tissue and blood was assayed following the procedure of Sinha.[24] Measurement of lipid peroxidation was done by the method described by Ohkawa et al.[25] The amount of TBARS was calculated using a molar extinction coefficient of 1.56×10^5/M/cm. In case of blood, the absorbance of supernatant was read at 532 nm and the values were expressed as moles of MDA/ml.

Activities of AST and ALT were determined by colorimetric assay using a commercial kit based on IFCC (Ecoline ALAT; Catalog No. A2320309, Merck, Germany; Ecoline ASAT; Catalog No. A2340109, Merck, Germany) following manufacturer's protocol.

Statistical analysis

The results are expressed as the mean ± SEM of number of observations. Comparisons of means were carried out using one way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare means between the different treatment groups. Differences were considered significant at $P < 0.05$ unless otherwise stated in the text.

RESULTS

Effect of nanoparticle and antioxidants on body weight

Significant reduction in body weight was observed in mice exposed to Al_2O_3 nanoparticles for 7 days (table 1). No changes were observed in groups receiving both NP and antioxidants simultaneously.

Effect of nanoparticle and antioxidants on heme synthesis pathway

Significantly altered activity of ALAD was observed in the nanoparticle exposed group compared to normal (Figure 1). A marked recovery was observed in the group co-exposed to nanoparticle and alpha lipoic acid. Quercetin administration was less effective than alpha lipoic acid in reducing ALAD inhibition.

Table 1 Effect of alpha lipoic acid and quercetin on body weight changes on Al_2O_3 NP exposure in mice

Group	Initial body weight (in g)	Final body weight (in g)	Body weight gain or loss (in g)
Normal animal	24.90 ± 0.11	25.20 ± 0.10	+0.30 ± 0.003
Al_2O_3 NP	25.10 ± 0.12	23.70 ± 0.11	-1.40 ± 0.005
Quercetin	25.80 ± 0.10	26.20 ± 0.14	+0.40 ± 0.005
Alpha lipoic acid	24.90 ± 0.11	25.50 ± 0.13	+0.50 ± 0.003
NP + Quercetin	25.20 ± 0.13	23.90 ± 0.09	-1.30 ± 0.005
NP + Alpha lipoic acid	25.00 ± 0.12	24.10 ± 0.11	-0.90 ± 0.003

Values are mean ± SE; n=4.

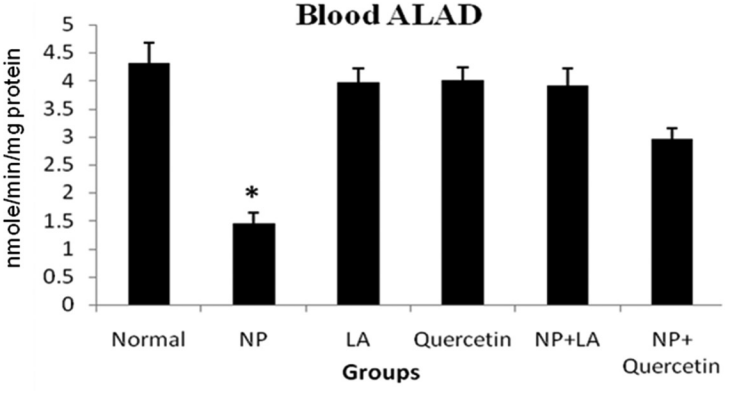

Figure 1. Effect of alpha lipoic acid and quercetin on blood ALAD activity after Al_2O_3 NP exposure in mice.

* P<0.05 compared to normal animals; † P< 0.01; ‡ P < 0.05 compared to Al_2O_3 nanoparticle exposed group.

Effect on blood oxidative stress variables

Significant elevation in ROS noted following oral Al_2O_3 exposure compared to normals. Treatment with quercetin or alpha lipoic acid slightly increased ROS. A significant recovery was observed following co-adminstration of alpha lipoic acid while Quercetin was not effective (Table 2). A significant elevation in TBARS was observed, which was effectively reduced by alpha lipoic acid (Table 2). Quercetin on the other was ineffective.

Effect on blood antioxidant status

A significant decrease in blood GSH, no change in SOD while an increased catalase activity was observed on NP exposure (Table 2). Alpha lipoic acid restored these alterations whereas quercetin was ineffective.

Effect on tissue oxidative stress

Liver ROS and TBARS increased on Al_2O_3 exposure compared to normal (Table 3). Alpha lipoic acid was most effective than quercetin in reducing ROS while no effects of antioxidants on TBARS was noted.

Effect on antioxidant potential in liver

GSH: GSSG ratio, catalase and SOD activities were significantly lowered in case of Al_2O_3 NP exposure (Table 3). Alpha lipoic acid was most effective than quercetin in restoring catalase, SOD activities and GSH: GSSG ratio towards norma, (Table 3).

Effect on plasma transaminases indicative of hepatic damage

GOT and GPT activities increased on Al_2O_3 NP exposure (Figure 2) which responded favorably to alpha lipoic acid and quercetin. Alpha lipoic acid was again found to attenuate NP induced alteration in the activities of these enzymes more effectively, compared to quercetin.

DISCUSSION

Natural antioxidants are better alternatives than synthetic antioxidants in counteracting various free radicals associated diseases and other pathological conditions.[6] Quercetin and lipoic acid possess the ability to overcome enhanced

Table 2 Effect of alpha lipoic acid and quercetin on blood biochemical variables on Al_2O_3 NP exposure in mice

Blood	Normal	Al_2O_3 NP	Lipoic acid (LA)	Quercetin	NP + LA	NP + Quercetin
ROS (FIU)	433.4 ± 12.7	549.8 ± 13.6*	435.5 ± 15.8	451.4 ± 23.5	430.2 ± 11.6†	445.2 ± 13.8†
GSH (mg/ml RBC)	5.22 ± 0.12	4.57 ± 0.07*	4.80 ± 0.05	4.31 ± .07	5.01 ± 0.1†	4.94 ± 0.21
TBARS (µg/ml RBC)	21.2 ± 0.5	30.4 ± 0.9*	20.2 ± 0.8	26.2 ± 0.8	21.1 ± 0.45†	24.6 ± 0.50‡
Catalase (nmoles of H_2O_2 consumed min^{-1} mg $protein^{-1}$	0.42 ± 0.03	0.61 ± 02*	0.47 ± .01	0.6 ± 0.01	0.46 ± 0.01†	0.53 ± 0.03‡
SOD (units min^{-1} mg $protein^{-1}$)	0.39 ± 0.03	0.41 ± 0.03	0.45 ± 0.02	0.59 ± 0.01	0.51 ± 0.01‡	0.49 ± 0.02

Values are mean ± SE; n=4.
* P<0.001 compared to normal animals; † P<0.001; ‡ P<0.05 compared to Al_2O_3 nanoparticle exposed group.

Table 3 Effect of alpha lipoic acid and quercetin on liver biochemical variables on Al_2O_3 NP exposure in mice

Liver	Normal	Al_2O_3 NP	Lipoic acid (LA)	Quercetin	NP + LA	NP + Quercetin
ROS (FIU)	334.2 ± 6.9	461.8 ± 15.7*	313.6 ± 7.6	330 ± 8.6	321.2 ± 3.5†	325.2 ± 10.6†
GSH/GSSG	1.23 ± 0.14	0.67 ± 0.15*	1.1 ± 0.12	0.98 ± 0.54	1.2 ± 0.91†	0.94 ± 0.11†
Catalase (nmoles of H_2O_2 consumed min^{-1} mg $protein^{-1}$	1.76 ± 0.31	0.76 ± 0.02*	0.67 ± 0.12*	0.69 ± 0.14*	1.21 ± 0.39‡	0.98 ± 0.34
SOD (units min^{-1} mg $protein^{-1}$)	1.39 ± 0.29	0.61 ± 0.08*	1.01 ± 0.2	0.99 ± 0.29	1.23 ± 0.32†	1.11 ± 0.36‡
TBARS (µg/g)	17.1 ± 1.4	27.41 ± 0.99*	21.55 ± 1.1*	25.55 ± 0.3	21.04 ± 0.67‡	26.83 ± 0.98

Values are mean ± SE; n=4.
* P<0.001 compared to normal animals; † P<0.001; ‡ P<0.05 compared to Al2O3 nanoparticle exposed group.

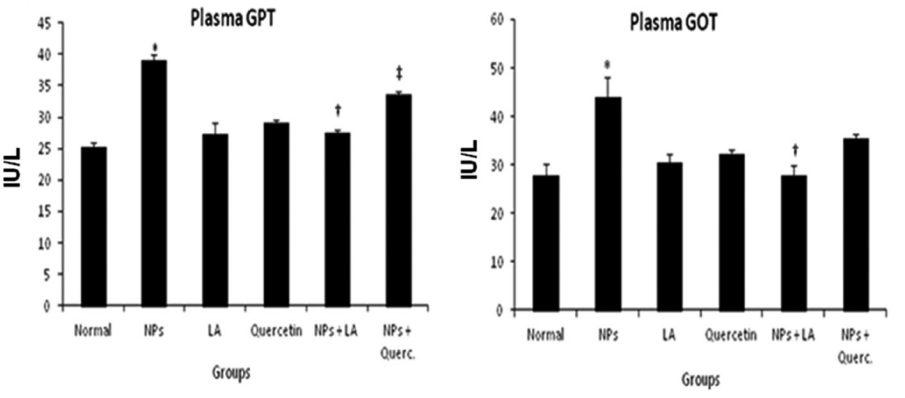

Figure 2. Effect of alpha lipoic acid and quercetin on plasma transaminases activities after Al_2O_3 NP exposure in mice.
* P<0.05 compared to normal animals; † P< 0.01; ‡ P < 0.05 compared to Al_2O_3 nanoparticle exposed group.

oxidative stress conditions and hence protects the cell and tissues from oxidative damage.[14] Nanoparticles exert their toxic effects through the generation of various deleterious free radicals including, ROS like hydrogen peroxide, hydroxyl radical species, nitric oxide or superoxide anion.[26] Hence, generation of free radicals results in imbalance between pro-oxidant and antioxidants leading to oxidative injury which can be effectively prevented by the simultaneous supplementation of antioxidants.[13–14] We selected two natural antioxidants, alpha lipoic acid and quercetin for their protective efficacy against Al_2O_3 nanoparticles induced hepatotoxicity in this study.

Oral Al_2O_3 nanoparticles exposure led to the biochemical alterations in blood and liver of mice. Though nanoparticles possess different routes for their entry into the body. Erythrocytes or Red Blood Cells being most dominant cells in the body are more vulnerable to the toxic effect by these nanoparticles.[27] Due to their small size and enhanced absorption properties, nanoparticles can easily interact with their membrane causing its agglutination by changing cell membrane structure and properties.[27] Heme biosynthesis is a critical pathway for all mammals and is known to be highly susceptible to alteration induced by metals.[14] ALAD is a zinc dependent metalloenzyme, reported to play a key role in heme biosynthesis.[28] Till now, effect of nanoparticle toxicity on δ-ALAD enzyme has not been reported. We observed significant ALAD inhibition in the group exposed to NP alone. Alpha lipoic acid was most effective than quercetin in restoring blood ALAD activity. Inhibition of ALAD results in the accumulation of its substrate δ-ALA which further gets rapidly oxidized to generate free-radicals/ ROS which in term could explain the toxicity produced by nanoparticles.[28,29] Lipoic acid possess better ability to chelate metal ions thus, reducing the concentration of metal ions in the blood stream and resulting in decreased competition between Zn, a cofactor of ALAD enzyme and aluminum.[30]

Cellular oxidative stress was evident by elevated ROS level, reduced glutathione level, increased lipid peroxidation and impaired antioxidant defense status. Our study reported elevated ROS levels in Al_2O_3 nanoparticle-treated group, suggesting free radical generation leading to oxidative stress conditions. Increased lipid peroxidation like elevated TBARS and reduced GSH, further signifies oxidative stress condition. Concomitant administration of antioxidants particularly alpha-lipoic acid led to pronounced recovery, suggesting it be a more effective scavenger of free radicals than quercetin. ROS generation leads to impaired cellular antioxidant defense system. GSH levels decreased after NP treatment, possibly the to its increased utilization in neutralizing free radicals. Glutathione is the major form of cellular glutathione and earlier reports support our results for the protective efficacy of alpha-lipoic acid and quercetin against environmental toxicants induced GSH depletion.[13,31] There have been few conflicting reports in the past about prooxidant potential of quercetin or its GSH inhibitory activity.[32] This is in agreement with our results obtained in blood, although results in liver showed antioxidant potential. One of the possible explanation for this could be that only a small portion of quercetin enters the blood stream and exhibit prooxidant activity while the major concentration is known to get metabolized by liver.[6] Elevated ROS levels are implicated in the damage of biological molecules such as lipids, which are altered by peroxidation. Elevation in TBARS is an indicator of lipid peroxidation under oxidative stress condition.[14] We observed a significant elevation in blood and hepatic TBARS level following NP exposure. Interestingly the level of blood and hepatic

TBARS, plasma GOT and GPT were restored to normal in the animals co-exposed to NP and alpha lipoic acid particularly.[33]

We also determined the antioxidant profile. SOD prevents the harmful effects of superoxide ion by converting them into less toxic hydrogen peroxides which subsequently splits into non toxic water and oxygen molecule by catalase activity.[6] Catalase is another major antioxidant enzyme whose activity decreases during oxidative stress, leading to H_2O_2 accumulation and finally peroxidation of lipids.[6] We observed decreased hepatic SOD activity in our study. SOD is one of the components of intrinsic antioxidant defense system, and is responsible for dissemination of Superoxide radicals. During oxidative stress the body uses its defense mechanism to minimize the process of lipid peroxidation by using the antioxidant enzymes such as SOD, thus, the activity of this enzyme become higher in early stages of insult, but if the insult continue, the enzyme become depleted which means that in advance stages of peroxidation the activity of SOD declined. We observed a significant decrease in hepatic SOD and Catalase activity on NP exposure which responded favorably to the co-administration of antioxidants. Apart from these two enzymes, we also observed a significant decrease in liver GSH:GSSG, considered to be a crucial biomarker of oxidative stress.[17] Significant decrease in GSH: GSSG ratio in NP exposed animals suggest possible involvement of nanoparticles in decreasing GSH concentration. However, the depletion of GSH: GSSG ratio was significantly protected by alpha -lipoic acid and quercetin suggesting their antioxidant properties.

Among the two antioxidants, co- administration of alpha-lipoic acid with Al_2O_3 nanoparticles was found to be most effective in reducing nanoparticle induced inhibition of blood ALAD activity. The elevation in levels of ROS in both blood and liver responded favorably to alpha lipoic acid compared to quercetin. Also inhibited levels of antioxidant enzymes and GSH were significantly protected by co-administration of alpha lipoic acid. In our study we used alpha- lipoic acid which is the racemic mixture of R-lipoic acid and S-lipoic acid and it gets readily converted into its reduced form DHLA.[34] Both ALA and DHLA are powerful antioxidants and hence better results were observed compared to quercetin. Their antioxidant functions involves: (i) quenching of reactive oxygen species; (ii) regeneration of endogenous and exogenous antioxidants involving vitamins C and E and glutathione; (iii) chelation of redox metals including Cu (II) and Fe (II); (iv) repair of oxidized proteins.[32] The effective implementation of all these functions is clearly evident from our results. The DHLA/ALA couple has a redox potential of −320 mV, and hence DHLA has one of the highest antioxidant potentials known in biological systems.[15]

It thus can be assumed that the antioxidant efficacy of quercetin may be due to (i) its higher diffusion rate into the membranes allowing to scavenge free radicals at various sites; (ii) its pentahydroxyflavone structure, allowing to chelate metal ions[6,32]; (Figure 3) (iii) regeneration of endogenous and exogenous antioxidants involving vitamin C and E and glutathione and (iv) presence of sulfhydryl group in the structure justified its use in this study against nanometallic toxicity. We however found better efficacy of lipoic acid over quercetin which could be due to i) Its chelating property i.e., absorption into the intracellular environment and complexing metals previously bound to other sulfhydryl proteins, ii) LA in unbound form is chemically able to trap circulating metals, thus preventing cellular damage caused by metal toxicity and iii) LA being lipophilic is able to penetrate cell membranes and reach high intracellular concentrations immediately on administration. Moreover the relatively good scavenging activity of lipoic acid is due to the strained conformation of the 5-membered ring in the intramolecular disulfide[32] (Figure 3). Combining results of the study, we may not exclude the possibility of a decreased oxidative stress when these natural antioxidants were employed for the protection of toxic manifestations. However, further experimentation are needs required to investigate a possible mechanism by which these natural antioxidants effectively NP induced toxicity.

CONCLUSION

In conclusion, our study confirms that nanoparticles upon reaching systemic circulation and target organs may produce cellular injury if the magnitude of ROS production overwhelms the antioxidant defense status of the cell. However the role of antioxidants like alpha

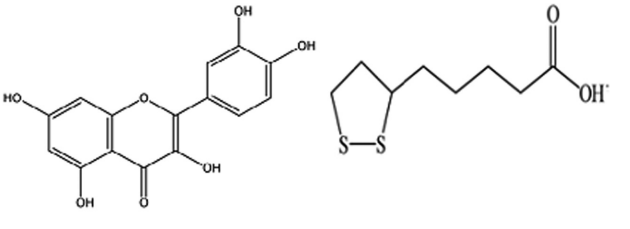

Figure 3. Structure of (A) Quercetin (B) Alpha lipoic acid.

lipoic acid may be beneficial in reducing the altered biochemical variables, suggestive of oxidative stress. Also, the study revealed alpha lipoic acid as the more effective antioxidant, followed by quercetin, as evident from the observed results. These results also point to moderate chelating property of alpha lipoic acid and quercetin against Al_2O_3 nanoparticles. These flavonoids may also be co-supplemented during chelation treatment, thereby achieveing a enhanced hepatoprotective effect against nanoparticle-induced cytotoxicity.

CONFLICT OF INTEREST

All authors have none to declare.

ACKNOWLEDGEMENT

Authors thank Director of the Establishment for his support and encouragement. Rupal Shrivastava thanks M.P. Council of Science and Technology (MPCOST) for the award of a Junior Research Fellowship.

REFERENCES

1. Borm PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, et al. The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol. 2006; 3:11.
2. Prabhakar PV, Reddy UA, Singh SP, Balasubramanyam A, Rahman MF, Indu Kumari S, et al. Oxidative stress induced by aluminum oxide nanomaterials after acute oral treatment in Wistar rats. J Appl Toxicol. 2012; 32:436–45.
3. Campbell A, Becaria A, Lahiri DK, Sharman K, Bondy SC. Chronic exposure to aluminum in drinking water increases inflammatory parameters selectively in the brain. J Neurosci Res. 2004; 75:565–72.
4. Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nano level. Science. 2006; 311:622–7.
5. Schrand AM, Rahman MF, Hussain SM, Schlager JJ, Smith DA, Syed AF. Metal-based nanoparticles and their toxicity assessment. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010; 2:544–68.
6. Flora SJS, Srivastava R, Mittal M. Chemistry and pharmacological properties of some natural and synthetic antioxidants for heavy metal toxicity. Current Med. Chem 2013; 20:4540–74.
7. Hendriks JJA, de Vries HE, van der Pol SMA, van den Berg TK, van Tol EAF, Dijkstr CD. Flavonoids inhibit myelin phagocytosis by macrophages; a structure–activity relationship study. Biochem Pharmacol. 2003; 65: 877–85.
8. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002;13: 572–84.
9. Park C, So H, Shin C, Baek S, Moon B, Shin S, et al. Quercetin protects the hydrogen peroxide-induced apoptosis via inhibition of mitochondrial dysfunction in H9c2 cardiomyoblast cells. Biochem Pharmacol. 2003; 66: 1287–95.
10. Negre-Salvagyre A, Salvagyre R. Quercetin prevents the cytotoxicity of oxidized LDL on lymphoid cell lines. Free Radic Biol Med. 1992; 12:101–6.
11. Makita H, Tanaka T, Fujituka H, Tatematsu N, Satoh K, Harah A, Mori, H et al. Chemoprevention of 4-nitroquinoline 1-oxide-induced rat oral carcinogenesis by the dietary flavonoids chalcone, 2-hydroxychalcone, and quercetin. Cancer Res. 1996; 59:4904–9.
12. Packer L, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition. 2001; 17:888–95.
13. Mishra D, Flora SJS. Quercetin administration during chelation therapy protects arsenic-induced oxidative stress in mice. Biol Trace Elem Res. 2008; 7:8064–9.
14. Bhatt K, Flora SJS. Oral co-administration of α -lipoic acid, quercetin and captopril prevents gallium arsenide toxicity in rats. Environ Toxicol Pharmacol. 2009; 28:140–46.
15. Domitrovic R, Jakovac H, Vasiljev Marchesi V, Vladimir-Knezevic S, Cvijanovic O, Tadic Z, et al. Differential hepatoprotective mechanisms of rutin and quercetin in CCl(4)-intoxicated BALB/cN mice. Acta Pharmacol Sin. 2012; 33(10):1260–70.
16. Ercal N, Treeratphan P, Hammond TC, Matthews RH, Grannemann NH, Spitz DR. In vivo indices of oxidative stress in lead-exposed C57BL/6 mice are reduced by treatment with meso-2, 3-dimercaptosuccinic acid or N-acetylcysteine. Free Radical Biol Med. 1996; 21(2):157–61.
17. Zhang QL, Li MQ, Ji JW, Gao FP, Bai R, Chen CY et al. In vivo toxicity of nano-alumina on mice neurobehavioral profiles and the potential mechanisms. Int J Immunopathol Pharmacol. 2011; 24(1 Suppl): 23S–29S.
18. Steck TL, Kant JA. Preparation of impermeable ghosts and inside-out vesicles from human erythrocyte membranes. Method Enzymol. 1974; 31: 172–80.
19. Socci DJ, Bjugstad KB, Jones HC, Pattisapu JV, Arendash GW. Evidence that oxidative stress is associated with the pathophysiology of inherited hydrocephalus in the H-Tx rat model. Exp Neurol. 1999; 155:109–17.
20. Berlin, A., Schaller, K.H., 1974. European standardized method for the determination of delta aminolevulinic acid dehydratase activity in blood. Zeit. Klin. Chem. Klin. Biochem. 12:389–90.
21. Jollow DJ, Mitchell JR, Zamppaglione Z, Gillette JR. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolites. Pharmacol. 1974; 11:51–7.
22. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide-dismutase. Ind J Biochem Biophys. 1984; 21:130–32.
23. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972; 47: 389–94.
24. Ohkawa H, Onishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem.1979; 95:351–8.
25. Yan L, Gu Z, Zhao Y. Chemical mechanisms of the toxicological properties of nanomaterials: Generation of intracellular reactive oxygen species. Chem Asian J. 2013.
26. Li SQ, Zhu RR, Zhu H, Xue M, Sun XY, Yao SD, et al. Nanotoxicity of TiO2 nanoparticles to erythroctyte in vitro. Fd Chem Toxicol. 2008; 46: 3626–31.
27. Yusof M, Deniz Y, Ercal N. N-acetyl- L - cysteine protects against delta aminolevulinic acid induced 8-hydroxyguanosine formation. Toxicol Lett. 106:41–7.
28. Dhakshinamoorthy A, Navalon S, Alvaro M, Garcia H. Metal nanoparticles as heterogeneous Fenton catalysts. ChemSusChem. 2012; 5(1):46–64.
29. Vieria VLP , Rocha JBT , Schetinger MRC, Morsch VM, Rodrigues RR, Tuerlinchz SM, et al. Effect of aluminium on δ-aminolevulinic acid dehydratase from mouse blood. Toxicol Lett. 2000; 117(1–20):45–52.
30. Ramos AM, Aller P. Quercetin decreases intracellular GSH content and potentiates the apoptotic action of the antileukemic drug arsenic trioxide in human leukemia cell lines. Biochem Pharmacol. 2008; 75: 1912–23.
31. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. Gen Pharmacol. 1997; 29:315–31.
32. Flora SJ. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxid Med Cell Longev. 2009; 2(4):191–206.
33. Bast A, Haenen GR. Lipoic acid: a multifunctional antioxidant. Biofactors. 2003; 17:207–13.
34. Flora SJS. Arsenic induced oxidative stress and its reversibility. Free Radical Biol Med. 2011; 51:257–28.