SECTION 4: ANTIOXIDANT ACTIVITY OF ALPHA LIPOIC ACID AND QUERCETIN

Aluminum oxide (Al₂O₃) nanomaterial is an important nanoparticle due to its promising and everyday applications such as, specific drug delivery, explosives, ammunition, etc. However indiscriminate use of nano aluminium may release oxidized form of nano Al₂O₃ into the environment finally resulting in genetic damage, carcinogenicity, cytotoxicity [21, 28, 34]. Once the particle enters systemic circulation, it may affect sensitive target sites such as brain, bone marrow, lymph nodes, spleen, and heart [16, 227]. This necessitates the study of environmental impact and toxicity of nanoparticles and also the development of effective preventive and therapeutic strategies. Supplementation of natural antioxidants is one such strategy. Flavonoid includes various classes such as flavones, flavanones, and isoflavones. Various beneficial activities of flavonoids have been identified such as antioxidant, antitumor, and anti-inflammation activity [131, 132, 133].

Quercetin and other flavonoids such as alpha lipoic acid have been shown to possess broad range of pharmacological properties, such as carcinostatic activity, suppression of cell proliferation and tumour formation, prevention of platelet aggregation, stabilization of immune cells, and relaxation of cardiovascular smooth muscle [228, 137]. Quercetin however 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. Intake of quercetin rich diet was reported to inhibit the development of rat mammary cancer, colonic neoplasia and oral carcinogenesis [229, 230]. Apart from quercetin, lipoic acid is another potent antioxidant implicated in the treatment of a variety of diseases, like, liver cirrhosis, heavy metal poisoning, and diabetic polyneuropathy [140]. Such desirable properties of alpha-lipoic acid and quercetin justify their use in the present study against nanoparticle toxicity. This study was planned to investigate the comparative efficacy of alpha- lipoic acid and quercetin. Since most of the toxic chemicals are metabolized in liver, there is a high risk of free radical attack, which in turn leads to hepatotoxicity, and development of severe pathological conditions. Thus liver has been selected as the major target organ for this study.
**Animals and treatment**

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

- **Group I:** Normal Animal (received normal water)
- **Group II:** Al$_2$O$_3$ nanoparticles (100 mg/kg b.w.) oral, once, daily
- **Group III:** Quercetin (50mg/kg b.w.) oral, once, daily
- **Group IV:** Alpha-lipoic acid (50mg/kg b.w.) oral, once, daily
- **Group V:** Quercetin (50mg/kg b.w) + Al$_2$O$_3$ NPs (100 mg/kg b.w.)
- **Group VI:** Alpha lipoic acid (50mg/kg b.w) + Al$_2$O$_3$ NPs (100 mg/kg b.w.)

Antioxidants were administered after an interval of 4 hrs from the oral dosage of nanoparticles. Doses of antioxidants and nanoparticles were selected on the basis of earlier publications [231] [232] [233]. We selected oral route of exposure for rats 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. They may also enter into the gastrointestinal tract after their accidental release into the environment. After 7 days of exposure, animals were sacrificed under light ether anesthesia, 48 h, after last dosing. Blood was collected in heparinized vial and liver was collected and stored at -80°C. For biochemical estimation, tissues were washed with cold normal saline, blotted and all extraneous materials were removed.

**Biochemical assays**

RBCs were isolated by the method of Steck and Kant (1974) [184]. The packed cell volume (PCV) was divided into two parts. First part was diluted with chilled distilled water and kept for the analysis of reactive oxygen species (ROS), catalase, thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH). Other part was used for the estimation of antioxidant enzymes like superoxide dismutase (SOD). Amount of ROS in blood was measured using 2’, 7’-dichlorofluorescein diacetate (DCF-DA) dye. DCF-DA gets converted into highly fluorescent DCF by cellular peroxides (including hydrogen peroxide). The methodology used was described by Socci et al., (1999) [169]. The activity of blood δ-aminolevulinic acid dehydratase (ALAD) was assayed according to the method provided by Berlin and Schaller (1974) [162]. Analysis of blood GSH
concentration was performed by the method described by Ellman, (1959) [160] and modified by Jollow et al., (1974) [161]. Superoxide dismutase (SOD) activity was quantified spectrophotometrically by the method of Kakkar et al., (1984) [167]. Catalase activity in tissue and blood was assayed following the procedure of Sinha (1972) [168] at room temperature. Measurement of lipid peroxidation in the form of TBARS was done by the method given by Ohkawa et al (1971) [163] in tissue and Stock and Dormandy (1979) [224] in blood. 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.

Results

Effect of nanoparticle and antioxidants on body weight

Table 4.6 shows the effect of NP exposure on body weight in mice. Significant reduction in body weight was observed in mice exposed to Al₂O₃ nanoparticles for 7 days. However no changes were observed in groups exposed to quercetin and alpha lipoic acid alone. No significant changes or reduction was also observed in groups receiving both NP and antioxidants simultaneously.

Table 4.6: Effect of alpha lipoic acid and quercetin on body weight changes on Al₂O₃ NP exposure in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Body weight gain or loss (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal animal</td>
<td>24.90 ± 0.11</td>
<td>25.20 ± 0.10</td>
<td>+0.30 ± 0.003</td>
</tr>
<tr>
<td>Al₂O₃ NP</td>
<td>25.10 ± 0.12</td>
<td>23.70 ± 0.11</td>
<td>-1.40 ± 0.005*</td>
</tr>
<tr>
<td>Quercetin</td>
<td>25.80 ± 0.10</td>
<td>26.20 ± 0.14</td>
<td>+0.40 ± 0.005</td>
</tr>
<tr>
<td>Alpha lipoic acid</td>
<td>24.90 ± 0.11</td>
<td>25.50 ± 0.13</td>
<td>+0.50 ± 0.003</td>
</tr>
<tr>
<td>NP + Quercetin</td>
<td>25.20 ± 0.13</td>
<td>23.90 ± 0.09</td>
<td>-1.30 ± 0.005</td>
</tr>
<tr>
<td>NP + Alpha lipoic acid</td>
<td>25.00 ± 0.12</td>
<td>24.10 ± 0.11</td>
<td>-0.90 ± 0.003</td>
</tr>
</tbody>
</table>

Values are mean ± SE; n=4
Effect of nanoparticle and antioxidants on blood ALAD activity

Figure 4.21 illustrates the effect of Al$_2$O$_3$ nanoparticle on blood ALAD activity. ALAD is an important biomarker for the evaluation of altered heme biosynthetic pathway and is found to be susceptible to various environmental pollutants particularly metals. Significantly altered ALAD activity was observed in the nanoparticle exposed group compared to normal. No such inhibition was noted in groups exposed to antioxidants (quercetin and alpha lipoic acid). A marked recovery was also observed in the group co-exposed to nanoparticle and alpha lipoic acid. Quercetin did exhibit significant recovery; however the recovery is less compared to alpha lipoic acid.

![Blood ALAD Graph](image)

**Figure 4.21**: Effect of alpha lipoic acid and quercetin on blood ALAD activity after Al$_2$O$_3$ NP exposure in mice.

**Abbreviation used and unit**: ALAD; Delta Amino Levulinic Acid Dehydratase as nmole/min/mg protein. Values are mean±SE; n=4. *P=0.05 compared to normal animals.

Effect on blood oxidative stress variables

Enhanced generation of free radicals is the most important biomarker of oxidative stress, which signifies potential cell or tissue injuries. Significant elevation in the levels of ROS was noted following oral exposure to Al$_2$O$_3$ nanoparticles compared to normal. Animals treated with quercetin or alpha lipoic acid alone shows a moderate increase in
ROS generation, however the change was insignificant. A significant recovery was observed following co-administration of alpha lipoic acid that signifies effective neutralization of free radicals. Quercetin was not effective and only provided moderate restoration (Table 4.7). On nanoparticles exposure significant elevation TBARS level was observed, which was effectively reduced by alpha lipoic acid (Table 4.7). Quercetin on the other hand did not effectively lower lipid peroxidation.

**Effect on Blood antioxidant status**

Effect of Al₂O₃ NPs on blood antioxidant variables is shown in Table 4.7. A significant decrease in blood GSH level was observed upon NP exposure. However no remarkable alteration in the SOD activity was observed. Catalase increased significantly on NP exposure. Alpha lipoic acid restored this alteration whereas quercetin was ineffective.

**Table 4.7: Effect of alpha lipoic acid and quercetin on blood biochemical variables on Al₂O₃ NP exposure in mice.**

<table>
<thead>
<tr>
<th>Blood</th>
<th>Normal</th>
<th>Al₂O₃ NP</th>
<th>Lipoic acid (LA)</th>
<th>Quercetin</th>
<th>NP + LA</th>
<th>NP + Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS</td>
<td>433.4± 12.7</td>
<td>549.8± 13.6*</td>
<td>435.5± 15.8</td>
<td>451.4± 23.5</td>
<td>430.2± 11.6†</td>
<td>445.2± 13.8†</td>
</tr>
<tr>
<td>GSH</td>
<td>5.22± 0.12</td>
<td>4.57± 0.07*</td>
<td>4.80± 0.05</td>
<td>4.31± .07</td>
<td>5.01± 0.1†</td>
<td>4.94± 0.21</td>
</tr>
<tr>
<td>TBARS</td>
<td>21.2± 0.5</td>
<td>30.4± 0.9*</td>
<td>20.2± 0.8</td>
<td>26.2±0.8</td>
<td>21.1± 0.45†</td>
<td>24.6± 0.50†</td>
</tr>
<tr>
<td>Catalase</td>
<td>0.42±0.03</td>
<td>0.61± .02</td>
<td>0.47±.01</td>
<td>0.6± 0.01</td>
<td>0.46± 0.01†</td>
<td>0.53± 0.03†</td>
</tr>
<tr>
<td>SOD</td>
<td>0.39±0.03</td>
<td>0.41±0.03</td>
<td>0.45±0.02</td>
<td>0.59±0.01</td>
<td>0.51±0.01†</td>
<td>0.49±0.02</td>
</tr>
</tbody>
</table>

**Abbreviations used and units:** ROS- Reactive Oxygen Species as Fluorescent Intensity unit (FIU); GSH as reduced Glutathione as mg/ml of RBCs; TBARS- Thiobarbituric Acid Reactive Substances as µg/gm of tissue weight, SOD: Superoxide Dismutase as units min⁻¹ mg protein⁻¹; Catalase as nmoles of H₂O₂ consumed min⁻¹ mg protein⁻¹. 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.

**Effect on tissue oxidative stress**

Effect of Al₂O₃ NPs on liver oxidative stress variables is shown in Table 4.8. Increased liver ROS was noted on exposure to Al₂O₃ NPs, compared to normal. However alpha lipoic acid was found to be much more effective than quercetin as it significantly neutralized deleterious free radicals and thus found to be better. Alpha lipoic acid or
quercetin when administered alone no effect of ROS was noted. In case of lipid peroxidation, elevated level of TBARS was observed in case of group exposed to Al$_2$O$_3$ NPs. However upon treatment with antioxidants, no significant recovery was observed.

**Effect on antioxidant potential in liver**

GSH: GSSG ratio was significantly lowered in case of Al$_2$O$_3$ NP exposure. Catalase and SOD activity decreased significantly compared to normal on NP exposure. Alpha lipoic acid was able to restore catalase and SOD activity and a decreased GSH: GSSG ratio towards normal, quercetin on the other hand was found to be less effective as only slight restoration was noted (Table 4.8). Thus alpha lipoic acid was found to be more effective in ameliorating NP induced oxidative stress compared to quercetin.

**Table 4.8: Effect of alpha lipoic acid and quercetin on liver biochemical variables on Al$_2$O$_3$ NP exposure in mice.**

<table>
<thead>
<tr>
<th>Liver</th>
<th>Normal</th>
<th>Al$_2$O$_3$ NP</th>
<th>Lipoic acid (LA)</th>
<th>Quercetin</th>
<th>NP +LA</th>
<th>NP +Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS</td>
<td>334.2± 6.9</td>
<td>461.8± 15.7*</td>
<td>313.6± 7.6</td>
<td>330± 8.6</td>
<td>321.2± 3.5†</td>
<td>325.2± 10.6†</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>1.23±0.14</td>
<td>0.67±0.15*</td>
<td>1.1± 0.12</td>
<td>0.98±0.54</td>
<td>1.2±0.91†</td>
<td>0.94±0.11†</td>
</tr>
<tr>
<td>Catalase</td>
<td>1.76±0.31</td>
<td>0.76±0.02*</td>
<td>0.67±0.12*</td>
<td>0.69±0.14*</td>
<td>1.21±0.39†</td>
<td>0.98±0.34</td>
</tr>
<tr>
<td>SOD</td>
<td>1.39±0.29</td>
<td>0.61±0.08*</td>
<td>1.01±0.2</td>
<td>0.99±0.29</td>
<td>1.23±0.32†</td>
<td>1.11±0.36‡</td>
</tr>
<tr>
<td>TBARS</td>
<td>17.1± 1.4</td>
<td>27.41± 0.99*</td>
<td>21.55± 1.1*</td>
<td>25.55± 0.3</td>
<td>21.04± 0.67†</td>
<td>26.83± 0.98</td>
</tr>
</tbody>
</table>

**Abbreviations used and units:** ROS- Reactive Oxygen Species as Fluorescent Intensity unit (FIU); GSH: GSSG ratio in mice liver. GSH, reduced glutathione as mg/gm tissue; GSSG, oxidized glutathione as mg/gm tissue; TBARS- Thiobarbituric Acid Reactive Substances as µg/gm of tissue weight, SOD: Superoxide Dismutase as units min−1 mg protein−1; Catalase as nmoles of H$_2$O$_2$ consumed min−1 mg protein−1. Values are mean ± SE; n=4. * P=0.001 compared to normal animals; † P=0.001; ‡ P=0.05 compared to Al$_2$O$_3$ nanoparticle exposed group.

**Effect on plasma transaminases indicative of hepatic damage**

Figure 4.22 shows significantly elevated activities of GOT and GPT on Al$_2$O$_3$ NP exposure. Antioxidants alpha lipoic acid and quercetin, significant restored the activity of these enzymes. Alpha lipoic acid was again found to attenuate NP induced alteration
in the activities of these enzymes more effectively, compared to quercetin, which was only partially effective the alteration.

**Figure 4.22: Effect of alpha lipoic acid and quercetin on plasma transaminases activities after Al\(_2\)O\(_3\) NP exposure in mice.**

**Abbreviations used and units:** GOT - Glutamate Oxaloacetate Transaminase as U/I; GPT, Glutamate Pyruvate Transaminase as U/I; Values are mean±SE; n=4. * P=0.05 compared to normal animals; † P=0.01 compared to Al\(_2\)O\(_3\) nanoparticle exposed group.

**Discussion**

Natural antioxidants are better alternatives than synthetic antioxidants in counteracting various free radicals associated diseases and other pathological conditions [234]. A variety of naturally occurring substances have been recognized to possess potential antioxidant abilities. In this study, we aimed at determining protective effects of alpha lipoic acid and quercetin against Al\(_2\)O\(_3\) nanoparticle induced oxidative stress in blood and hepatic tissues based on some selected biochemical variables. Scientific evidence supports the fact that few natural flavonoids like quercetin and lipoic acid possess the ability to overcome enhanced oxidative stress conditions and hence protects the cell and tissues from oxidative damage [141]. One of the main mechanism through which nanoparticles have been shown to exert their toxic effects is the generation of various deleterious free radicals including, ROS like hydrogen peroxide [235, 32]. Hence,
Antioxidant Activity of ALA and Quercetin

Evaluation of Toxicity of Metal Based Nanoparticles Using Mouse as In Vivo Model

generation of free radicals under various physiological and pathological conditions results in imbalance between pro-oxidant and antioxidants leading to oxidative injury [236]. However, reports indicate simultaneous supplementation of antioxidants can effectively lead to prevention of oxidative injuries [237, 141]. Keeping in mind this fact, we examined two known natural antioxidants, alpha lipoic acid and quercetin for their protective efficacy against Al₂O₃ nanoparticles induced hepatotoxicity.

Oral administration with 100 mg/kg of Al₂O₃ nanoparticles for 7 consecutive days led to the biochemical alterations in blood and liver of mice (Table 4.7 and 4.8). Though nanoparticles possess different routes for their entry into the body, once inside, they reach various target organs through systemic circulation. Erythrocytes or Red Blood Cells being most dominant cells in the body are more vulnerable to the toxic effect by these nanoparticles [236] (Table 4.7). Being nanosize, nanoparticles can easily interact with their membrane causing its agglutination by changing cell membrane structure and properties [36]. Heme biosynthesis is a critical pathway for all mammals and also highly susceptible to various toxicants particularly metals [141]. Delta-amino levulinic acid dehydratase, is a zinc dependent metalloenzyme, reported to play a key role in heme biosynthesis [238]. Till now, effect of nanoparticle toxicity on δ-ALAD enzyme has not been reported. Interestingly our results exhibit significant inhibition in the ALAD activity in-group exposed to NP alone. However alpha lipoic acid was much effective in restoring the inhibited activity compared to quercetin. It has been reported that inhibition of ALAD results in accumulation of its substrate delta-aminolevulinic acid (δ-ALA), which further rapidly oxidizes to generate free-radicals / reactive oxygen species. Blood ALAD activity was recovered in animals, co-exposed with alpha-lipoic acid and quercetin, with alpha lipoic acid more potent than querseitin (Fig. 4.21). In case of metal NPs, metallic ions released such as Al³⁺, Fe²⁺, Cu⁺, Mn²⁺, Cr⁵⁺ and Ni²⁺ contributes to the generation of ROS via the Fenton-type reaction [239] [240]. These results further suggest that lipoic acid possess better ability to chelate metal ions. This helps in reducing the concentration of metal ions in blood stream and hence a decreased competition between Zn, a cofactor of ALAD enzyme and aluminum [241, 242].

Cellular oxidative stress was evident by elevated ROS level, reduced glutathione level, increased lipid peroxidation and impaired antioxidant defense status. The present study demonstrated significantly elevated ROS levels in Al₂O₃ nanoparticle-treated group, suggesting that NPs be responsible for free radical generation leading to oxidative
stress conditions [34] (Table 4.8). In the event of excessive ROS production, increased lipid peroxidation was noted, like elevated TBARS and reduced GSH, signifying oxidative stress condition. However concomitant with administration of antioxidants particularly alpha-lipoic acid pronounced significant recovery, suggesting it be a more effective scavenger of free radicals than quercetin. Also, elevated levels of ROS leads to impaired antioxidant defense system. Results show decreased GSH level after NP treatment (Table 4.7 and 4.8). A possible explanation for this could be increased utilization of GSH in neutralizing free radicals. Glutathione is known to be a key regulator of the redox state of protein cysteinyi thiols. It is one of the major form of cellular glutathione and earlier reports support our finding that protective efficacy of alpha-lipoic acid and quercetin includes GSH depletion [243]. There have been few conflicting reports in the past about prooxidant potential of quercetin or its GSH inhibitory activity [244]. This is in agreement with our results obtained in blood, although results in liver showed antioxidant potential. Previous reports have shown that minor portion of quercetin entering the blood stream exhibit prooxidant activity while the major concentration is metabolized by liver and shows antioxidant activity. Elevated ROS levels are simultaneously been implicated in the damage of biological molecules such as lipids. Elevation in TBARS is a potent indicator of lipid peroxidation under conditions of oxidative stress [245]. Here, we observed a significant elevation in blood and hepatic TBARS level following NP exposure (Table 4.7 and 4.8). But sooner after therapy, level of blood and hepatic TBARS were restored to normal in animals co-exposed to NP and alpha lipoic acid quercetin. Alpha lipoic acid as evident, provided almost partial recovery than quercetin, which provides marginal recovery. These results can be attributed to the free radical scavenging activity of lipoic acid which also proved effective in restoring altered activities of GOT and GPT in plasma (Fig. 4.22).

One of the key feature of oxidative stress is the imbalance between prooxidant and antioxidant homeostasis. To further investigate mechanism responsible for NP induced oxidative stress we also determined the antioxidant profile. Superoxide dismutase and catalase are two main antioxidant enzymes in providing protection against oxidative stress [246]. SOD, on one hand prevents the harmful effect of superoxide ion. It converts them into hydrogen peroxides, which subsequently splits into non toxic water and oxygen molecule. This reaction is catalysed by catalase enzyme [246]. Activity of catalase decreases during oxidative stress, leading to H$_2$O$_2$ accumulation and
peroxidation of lipids [247]. We observed decreased hepatic SOD activity in our study (Table 4.8). During oxidative stress the body uses its defense mechanism to minimize the damage or injury thus, the activity of SOD become higher in early stages toxicant attack. In later stage, however, the enzyme gets depleted and cannot fight against free radicals. Thus in advance stages of peroxidation the activity of SOD declined. Interestingly in our study we found significant decrease in hepatic SOD and catalase activity on NP exposure; however its activity restored back to normal following co-administration of antioxidants (Table 4.8). In fact there was more pronounced restoration of SOD and catalase activity upon alpha lipoic acid administration, compared to quercetin. We also determined the alteration in GSH: GSSG ratio in the liver, which is considered to be a crucial biomarker of oxidative stress. Significant decrease in GSH: GSSG ratio suggests possible involvement of nanoparticles in reducing GSH concentration. However, depletion of GSH: GSSG ratio was significantly recovered by alpha-lipoic acid and quercetin, further indicating their antioxidant properties (Table 4.8).

Among two antioxidants studied in the present study, co-administration of alpha-lipoic acid along with Al₂O₃ nanoparticles was found to be more effective. It partially recovered 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 reduced activity of antioxidant enzymes and GSH were recovered partially 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 [150]. ALA and DHLA, both are known powerful antioxidants and hence better results were observed compared to quercetin. Their antioxidant functions involve: (i) quenching free radicals; (ii) regenerating endogenous and exogenous antioxidants such as vitamins C and E and glutathione; (iii) ability to chelate redox metals including Cu (II) and Fe (II); (iv) repairing oxidized proteins [236]. The effective implementation of all these functions is clearly evident from our results.

On the other hand antioxidant efficacy of quercetin may be owed to several other factors such as (i) higher membrane diffusion rates allowing scavenging of free radicals [159]; (ii) pentahydroxyflavone structure, which effectively allow chelation of metal ions [248] (iii) regeneration of endogenous and exogenous antioxidants and (iv) presence of sulfhydryl group. We however found better efficacy of lipoic acid over quercetin. Alpha
lipoic acid showed partial recovery of all variables and this could be attributed to various advantages over quercetin:

i) Absorption into the intracellular environment and complexing metals.

ii) LA in free form can trap circulating metals, thus preventing cellular damage

iii) Being lipophilic in nature, it is able to penetrate cell membranes and reach high intracellular concentrations immediately on administration.

Moreover the relatively good scavenging activity of lipoic acid can also be because of the strained conformation of the 5-membered ring in the intramolecular disulfide [247]. Combining all results of this 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 is required to investigate a possible mechanism by which these natural antioxidants effectively reduce NP induced toxicity.

This study concludes that aluminum oxide nanoparticles possess the ability to reach systemic circulation and target organ system. Administration of natural antioxidants like alpha lipoic acid and quercetin proved to be beneficial in the recovery of altered biochemical variables, which is the most novel finding. Partial recovery was observed in case of alpha lipoic acid, as greater therapeutic effect was seen, in comparison to quercetin, where only marginal recovery was observed. Effective chelating property was seen with alpha lipoic acid, since it is more hydrophilic and has the ability to reach intracellular spaces. Such flavonoids may also be co-supplemented during chelation treatment, thereby achieving good therapeutic effect against nanoparticle-induced cytotoxicity.