Effect of Curcumin and Nano-curcumin on Reduce Aluminum Toxicity in Rats

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Abstract: Aluminum is a ubiquitous toxic metal that mainly affects brain, bone, spleen, liver, kidney, hepatic hematopoietic system. This study investigated the effect of Curcumin and Curcumin Nanoparticles on reduced the toxicity of aluminum chloride in treated rats. Six groups of rats were used: (1) control; (2) curcumin-treated rats (15 mg curcumin /Kg BW; (3) Nano-curcumin -treated rats (15 mg Nano-curcumin /Kg BW); (4) aluminum chloride-treated rats (100 mg/kg BW); (5) AlCl₃-curcumin-treated rats (100 mg AlCl₃ and 15 mg curcumin/kg BW); (6) AlCl₃- Nano-curcumin -treated rats (100 mg AlCl₃ and 15 mg Nano-curcumin /kg BW). The treatment with AlCl₃ alone caused significant (P≤ 0.05) increased in liver and kidney functions of rats, while the oral intake of curcumin and Nano-curcumin eliminate the harmful effect of AlCl₃. On the same side, the treatment with AlCl₃ alone significantly (P≤ 0.05) increase the free radical level and decreased the activities of antioxidant enzymes in plasma, while the treatment with curcumin and Nano-curcumin reduced this increased in free radicals and increased the activities of antioxidant enzymes. These results confirmed that the curcumin and Nano-curcumin reduced the toxicity effect of AlCl₃ in rats; moreover, Nano-curcumin has a best biological and antioxidant activity than curcumin in healthy and AlCl₃-treated rats.

Keywords: Curcumin, Nano-curcumin, Aluminum Toxicity, Nano-Toxicology

1. Introduction

Curcumin is the most active polyphenol compound in the rhizome of turmeric (Curcuma longa). Curcumin is known as a strong anti-inflammatory, antioxidant, antimutagenic, wound healing, neuroprotective, hypoglycemic, antimicrobial, antitumor, tissue regenerating, anti-angiogenic, anti-metastatic [1]. In addition, curcumin exhibits many pharmacological activities against many chronic diseases such as type 2 diabetes, multiple sclerosis, rheumatoid arthritis, Alzheimer’s disease, suppresses thrombosis and inhibits human immunodeficiency virus (HIV) replication, enhances wound healing and protects against liver injury, anticancer (breast, colon, cervical, stomach, pancreatic and liver cancer) with low or no intrinsic toxicity to healthy cells [2]. The effectiveness of curcumin therapy, however, is limited by its low oral bioavailability caused by its poor solubility in the gastrointestinal fluid, rapid degradation at the intestinal pH and rapid metabolism [3]. An obvious process to improve the poor biopharmaceutical properties of curcumin is to improve its solubility in water using nanocarriers. Nanocarriers have a small size (typically 10-100 nm) and can, besides for solubilization. The Nano-sizing of curcumin particles increases their bioavailability and, therefore, their efficacy in a possible clinical application [4]. Aluminum (Al) is the third most abundant metal and constitutes about 8% of the earth crust [5]. Aluminum exposed through a wide range of household items, cooking utensils, food additives, water treatment, foods, packaging, vaccines, pharmaceuticals, and a numerous medication such as antidiarrheal agents, antacids,
phosphate binders, antiperspirants, buffer aspirin as well as a few, cosmetics are important sources of Al for humans [6, 7]. Today the daily ingested dose via drinking water is estimated to be around 160 µg aluminum/day, high as 3500-5200mg aluminum/day (i.e. 50 mg/kg/day-75 mg/kg/day) as a result of aluminum-based antacid consumption [8]. Aluminum is a ubiquitous toxic metal that mainly affects brain, bone, spleen, liver, kidney, hepatic hematopoietic system, central nervous, skeletal, hematopoietic systems, respiratory, immunologic system [6]. Aluminum has been shown to have the potential to be a toxicant to the Toxicity to system of Al is also drawn an attention recently [9]. Aluminium is neurotoxic in humans body and has been implicated in several neurological disorders, like Alzheimer’s disease, Parkinsonism and dialysis encephalopathy, microcyclic hypochromic anemia and osteomalacia, in renal osteodystrophy, anaemia, breast cancer and autoimmune (auto-inflammatory) syndrome induced by vaccination [10, 11]. It has been reported that Al-induced toxicity is associated with anemia in humans and in animals [12]. The objective of this study was to evaluate the effect of curcumin and Nano-curcumin on reduce the toxicity in aluminum-treated rats.

2. Materials and Methods

2.1. Material

Curcumin powder was obtained from Hi-Media Laboratories Ltd., Mumbai, India. Aluminum chloride was obtained from Loba Chemie, Mumbai, India (orally administered by 100 mg/kg, according to Chiroma et al. [13]. Reduced glutathione; 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB); Sulfosalicylic acid (SSA); and Thiobarbituric acid (TBA) were obtained from Sigma- Aldrich, Co, Saint Louis, USA. Urea and Creatinine kits were obtained from Biosystems, Spain; Bilirubin kit was obtained from Diamond, Germany; AST, ALT kits were obtained from QUIMICA CLINICA APLICADA, Spain; and ALP, ACP kits were obtained from Biodiagnostic, Egypt.

2.2. Methods

2.2.1. Synthesis and Characterization of Curcumin Nanoparticles

Curcumin solution (5 mg/ml) was prepared by dissolving 100 mg curcumin powder in 20 ml Dichloromethane. One ml of stock solution was added to boiling water (50 ml) in drop-wise manner under ultra-sonication condition with an ultrasonic power and frequency of 50 kHz. The solution was sonicated for 30 min. After sonication, the mixture was stirred at 800 rpm for about 20 min till the orange colored precipitate was obtained. Thereafter, supernatant was discarded and the pellet obtained was used for further biological study. The obtained particles were characterization using Infrared spectroscopy (FTIR-8400S, Shimadzu, Japan) and electron microscopy (JEOL JEM-1230, Japan).

2.2.2. Experimental Design

Forty-eight male albino rats (7 weeks old and 158-169 g weight) obtained from the Institute of Graduate Studies and Research (IGSR), Alexandria University, Egypt. The design of the experiment was approved by the local committee in Alexandria University, Egypt, according to the protocol conforms to the guidelines of the National Institutes of Health (NIH). were dwelled plastic cages (4 /cage) with sawdust bedding and maintained in an air-conditioned animal house at a controlled temperature (22±2°C) and relative humidity (60±10%) with a photoperiod of 12 h light/12 h dark [14]. After two weeks of acclimatization, the animals were divided into six equal groups, 8 animals in each group. Group 1 was used as a control. Group 2 was daily orally treated with curcumin by 15 mg/Kg BW in 0.1% DMSO [15]. Groups 3 was daily orally treated with Nano-curcumin 15 mg / kg BW in distilled water [15]. Group 4 was daily orally administered aluminum chloride 100 mg/kg BW in distilled water [13]. Groups 5 and 6 were treated with AlCl₃ solution (100 mg/kg BW) plus orally given curcumin and Nano-curcumin (15 mg / Kg BW) respectively. Animals were daily treated with the tested aluminum chloride, curcumin and Nano-curcumin juice for 30 day. All treatment was administered daily as an oral dose using a gastric tube.

2.2.3. Feed and Water Intake

Feed and water intake were recorded throughout the experimental period, and mean daily feed and water intake were determined.

2.2.4. Body Weight

At first and end of the experimental period, body weights of rats were recorded.

2.2.5. Hematological Parameters

At the end of the experimental period (after 30 days) rats were anesthetized with ether and sacrificed and the blood samples were collected in two tubes: one containing EDTA (anti-coagulant) and the other containing Heparin (anti-coagulant). Non coagulated blood by EDTA was tested shortly after collection by Particle counter (from ERMA INC.-Tokyo. Model PCE-210) for measuring total erythrocyte count (TEC) red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), platelets count (PLT), red cell distribution width (RDW), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH). Total leukocyte counts (TLC) white blood cell count (WBC), lymphocytes, segmented neutrophils, monocytes and eosinophils.

2.2.6. Blood Biochemical Parameters and Enzyme Activities

The other part of heparinized blood samples were placed immediately on ice. Plasma was obtained by centrifugation of samples at 4000 rpm for 20 min, and was stored at -80ºC until used for analyses.

(i). Kidney Function

kidney functions were evaluated by measuring of serum levels of blood urea according to Tabacco et al. [16], total
bilirubin concentration according to Burtis et al. [17] and creatinine concentration was measured by the method of Fabiny and Ertingshausen [18] using commercial Kits.

(ii). Liver Function
The activities of plasma alanine transaminase (ALT) and aspartate transaminase (AST) were assayed by the method of Reitman and Frankel [19]. Alkaline phosphatase (ALP) activity and Acid Phosphatase (AcP) were determined in plasma according to the method of Belfield and Goldberg [20]; and Kind and King [21], respectively.

2.2.7. Antioxidant Activity
Plasma free radical was measured by thiobarbituric acid-reactive substances (TBARS) assay based on the method of Tappel and Zalkin [22]. Superoxide dismutase (SOD) and Glutathione S-transferase (GST) activities in plasma were determined according to the method of Misra and Fridovich [23]; and Habig et al. [24], respectively. Meanwhile, the concentration of Glutathione reduced (GSH) was determined according to the method of Jollow et al. [25].

2.2.8. Statistical Analysis
The data were analyzed by a general linear model procedure of the Fisher’s protected least-significant difference (PLSD) test using SAS, 2004. The differences among the means of all treatments at the significance level of \( P \leq 0.05 \). Correlations were calculated using Pearson’s correlation coefficient [26].

3. Results and Discussion

3.1. Characterization of Nano Curcumin

3.1.1. FTIR Spectrum of Curcumin Nanoparticles Powder
FTIR spectrum of curcumin in DMSO (control) and Nano-curcumin (experimental) was recorded. In FTIR spectrum of Nano-curcumin, peaks were observed at 1633, 1442, 1153 and 1033 cm\(^{-1}\) (Figure 1).

![Figure 1. FTIR spectrum of curcumin nanoparticles.](image)

3.1.2. Scanning Electron Microscopy (SEM) of Curcumin Nanoparticles
Scanning Electron Microscopy (SEM) is a very helpful tool for explore morphology of nanoparticles powders. SEMimages of the nanoparticles prepared via wet chemical route. It is unclouded that the nanoparticles apparent by SEMimage consist of a number of crystallites with rutile structure. SEMimages of the Curcumin nanoparticles (Figure 2) show that they are approximately in globular form and sizes are various (<100nm).

![Figure 2. Scanning electron microphotographs (SEM) of curcumin nanoparticles.](image)

3.2. Changes in Feed and Water Intake, Initial and Final Body Weight, and Body Weight Gain
Data presented in Table 1 showed the effect of curcumin and Nano-curcumin on the body weight, body weight gain, feed intake and water intake of rats treated with aluminum chloride. No significant \( (P \geq 0.05) \) differences were found between all groups in all previous parameters. These results confirmed that there was no effect of Al Cl\(_3\) on body weight and feeding of rats. These results agree with Kumar [27] who reported that treatment with oral aluminum chloride resulted not affected on weight of adult rats when treated for 60 days. Meanwhile, Abubakar et al. [28] found that the treated with aluminum alone had a reduced rate of weight gain that was attributed to decreased food intake.
3.3. Effect of Curcumin, Nano-Curcumin on Hematological Parameters of Rats Treated with Aluminum Chloride

Effect of curcumin, Nano-curcumin on hematological parameters of rats treated with aluminum chloride was present in Table 2. The treatment with aluminum chloride alone caused significant (P ≥ 0.05) decrease in RBC, Hb%, RDW% and MCH of rats, while, the treatment with curcumin and Nano-curcumin reduced the decrease in these parameters. Concerning HT, PLT, MCV and MCHC, no significant (P ≥ 0.05) changes were found after treated with aluminum chloride alone, curcumin and Nano-curcumin alone or with aluminum chloride treatment in compared with control group. This result agrees with this of Mahieu et al. [12], he indicated that AI-induced toxicities are associated with anemia in humans and in animals. Moreover, plasma Fe levels were significantly lower in the Aluminum-treated rats than that in control. Zhang et al. [29] reported that the increase of Al levels in the blood could destroy the structure and disorder functions of red blood cells, and caused hematological diseases. The binding of Al with erythrocytes was much stronger than to serum proteins, the half retention time of Al in red blood cell is ranging from 20 to 40 days [30]. Moreover, aluminum chloride suppressed the immune function of red blood cells of mice [31]. Zhang et al. [32] found that AlCl3 unrest the structure of erythrocyte membrane and incourage hypertension in rat. Meanwhile, the treatment of rats with AlCl3 alone caused significant increase in monocytes %, while the oral intake of curcumin and Nano-curcumin not affected on monocytes % of AlCl3-treated rats (Table 3). On the other side, No significant (P ≥ 0.05) changes were found in WBC, Lymphocytes (%), Neutrophils (%) and eosinophils of all rats groups whether treated with AlCl3 alone or AlCl3 with curcumin and Nano-curcumin.

### Table 2. Effect of curcumin, nano curcumin on hematological parameters of rats treated with aluminum chloride.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
<th>Control</th>
<th>Curcumin</th>
<th>Nano-curcumin</th>
<th>AlCl3</th>
<th>AlCl3 + curcumin</th>
<th>AlCl3 + Nano-curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td></td>
<td>160±±7.6</td>
<td>158±±7.2</td>
<td>161±±7.0</td>
<td>169±±4.3</td>
<td>160±±8.9</td>
<td>160±±6.5</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td></td>
<td>181±±5.8</td>
<td>182±±4.1</td>
<td>183±±8.5</td>
<td>191±±4.3</td>
<td>184±±4.8</td>
<td>185±±9.5</td>
</tr>
<tr>
<td>Body weight gain (g/day)</td>
<td></td>
<td>0.702±±0.069</td>
<td>0.809±±0.049</td>
<td>0.733±±0.048</td>
<td>0.734±±0.0516</td>
<td>0.812±±0.0359</td>
<td>0.833±±0.04059</td>
</tr>
<tr>
<td>Feed intake (g/day/kg BW)</td>
<td></td>
<td>161±±6.6</td>
<td>165±±4.2</td>
<td>161±±3.5</td>
<td>166±±8.4</td>
<td>164±±8.4</td>
<td>168±±5.5</td>
</tr>
<tr>
<td>Water intake (ml/day/kg BW)</td>
<td></td>
<td>77.7±±3.31</td>
<td>75.3±±3.38</td>
<td>73.6±±3.12</td>
<td>78.7±±3.30</td>
<td>76.7±±2.89</td>
<td>76.6±±2.463</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 5 for each treatment group.

Mean values within a row not sharing a common superscript letter (a,b,c) were significantly different, p<0.05. *percentage of control group.

Values within a row not sharing a common superscript letter (a,b,c) were significantly different, p<0.05. RBC = red blood cells; Hb = hemoglobin; Ht = hematocrit; PLT = platelets count; RDW = red cell distribution width; MCV = mean cell volume; MCH = mean corpuscular hemoglobin and MCHC = mean corpuscular hemoglobin concentration.

*percentage of control group.
Table 3. Effect of curcumin, nano-curcumin on white blood cell count (WBC) and differential counts of rats treated with aluminum chloride.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
<th>Control (X10^3/µl)</th>
<th>Curcumin (X10^3/µl)</th>
<th>AlCl₃ (X10^3/µl)</th>
<th>AlCl₃ + curcumin (X10^3/µl)</th>
<th>AlCl₃ + Nano-curcumin (X10^3/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>Control</td>
<td>8.60±0.2000^a</td>
<td>8.35±0.709^a</td>
<td>9.20±0.456^a</td>
<td>8.95±0.430^a</td>
<td>8.80±1.329^a</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>Control</td>
<td>75.5±1.80^a</td>
<td>75.2±3.87^b</td>
<td>78.0±3.59^a</td>
<td>77.0±3.81^a</td>
<td>76.5±2.30^a</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>Control</td>
<td>19.4±1.50^b</td>
<td>19.2±1.24^b</td>
<td>19.4±1.72^a</td>
<td>19.3±1.16^b</td>
<td>19.3±1.16^b</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>Control</td>
<td>4.00±0.70^b</td>
<td>3.90±0.678^b</td>
<td>5.80±0.583^a</td>
<td>5.30±0.539^a</td>
<td>5.30±0.831^a</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>Control</td>
<td>3.20±0.148^a</td>
<td>3.22±0.174^a</td>
<td>3.00±0.158^a</td>
<td>3.02±0.049^a</td>
<td>3.04±0.172^a</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE; n = 5 for each treatment group.
Mean values within a row not sharing a common superscript letters (a, b) were significantly different, p<0.05.
*percentage of control group.

3.4. Kidney Functions of Rats

The effect of curcumin and Nano-curcumin on kidney function of rats treated with AlCl₃ was present in Figure 3. The treatment with AlCl₃ alone caused significant (P≤ 0.05) increased in urea, creatinine, and bilirubin of rats, while the oral intake of curcumin and Nano-curcumin eliminate the harmful effect of AlCl₃ on kidney functions of rats. The treatment with curcumin and Nano-curcumin not affected on kidney functions of healthy rats compared with control group. No significant (P≥ 0.05) differences were found in kidney functions between Aluminum groups treated with curcumin and Nano-curcumin. The increased in urea and creatinine levels is indicate protein catabolism and/or kidney dysfunction [33]. The last studies reported that curcumin has therapeutic potential against renal diseases and nephroprotective activity [34]. In addition, curcumin has been reported to exert nephroprotective effects on renal damage of several experimental models, such as those of cisplatin, acetaminophen, nephrectomy, gentamicin [34].
3.5. Liver Functions of Rats

Liver is the site that toxic compounds get transformed into less harmful products to reduce toxicity; this will damage hepatocytes and produce hepatotoxicity [35]. Toxicity to hepatic system of Al is also drawn an attention recently [9]. In this study the treatment with AlCl₃ alone significantly (P≤ 0.05) increased the ALT, AST, ALP and ACP values in blood plasma of rats (Figure 4). The treatment with curcumin and Nano-curcumin showed protective effect of the liver function whereas that treated or not treated with AlCl₃. The oral intake of curcumin and Nano-curcumin decreased the increase in ALT, AST, ALP and ACP values of rats treated with AlCl₃. The effect of curcumin on liver enzyme was similar that of Nano-curcumin whether in normal or Aluminum- treated rats. These results are agree with Xu et al. [35] who reported that AlCl₃ exposure increased serum AST and ALT activities and caused liver histopathological lesion. The previous studies suggested that when the liver is injured, AST and ALT increased and released into peripheral blood, thus serum AST and ALT are recognized as the principal indicators of liver injury [36]. The significant increase in serum AST and ALT, indicating that AlCl₃ exposure leads to increased permeability, damage and/or necrosis of hepatocytes [35]. In the same way, Turkez et al. [37] reporting that after the oral intake of AlCl₃ (34 mg/kg body weight), serum AST and ALT are increased. Rats treated with Curcumin-AlCl₃ and Nanocurcumin-AlCl₃ was comparable to the control group regarding ALT, AST, ALP and ACP. This result was agreed with Abdel-Wahhab et al. [38]. Those found the oral administration with curcumin and Nano-curcumin decreased the toxicity effect of Hg in the liver of rats.

![Figure 4. Effect of curcumin, nano curcumin on liver function of rats treated with aluminum chloride.](image)

3.6. Effect of Curcumin, Nano Curcumin on TBARS and Antioxidant Enzymes of Male Rats Treated with Aluminum Chloride

Oxidative stress is known as one of the important mechanisms of Al toxicity. Al induces oxidative stress through three pathways. First pathway, pro-oxidant activity, due to the formation of Al superoxide complex between Al₃⁺ and superoxide radical anion, promoting biological oxidation both in vitro and in vivo [39]. Second pathway, reducing ferric iron to ferrous iron, which would promote Fenton reaction in vivo systems, increasing the concentration of free radicals, such as hydroxyl radical [40]. Third pathway, Al facilitates the production of superoxide anion through non-iron driven biological oxidations involving the photochemical, enzymatic, and chemical and bio-molecular
The effect of oral intake of curcumin and Nano-curcumin on antioxidant activity in plasma of rats treated with AlCl₃ in additionally healthy rats was presented in (Figure 5). The treatment with AlCl₃ alone significantly (P≤ 0.05) increased TBARS values in rats plasma, while the treatment with curcumin and Nano-curcumin reduced the increase in TBARS in AlCl₃ groups. Also, the oral administration of healthy rats with curcumin and Nano-curcumin decreased TBARS values than control group. AlCl₃ treatment caused significant (P≤ 0.05) decrease in SOD, GST activities in plasma. The oral intake of curcumin and Nano-curcumin protected the activity of SOD and GST activities and reduced the decrease in AlCl₃ groups. Concerning GSH concentration in healthy and AlCl₃ groups, the results showed a significant (P≤ 0.05) decrease in GSH value after treated with AlCl₃. Curcumin and Nano-curcumin improvement GSH value in healthy rats compared to control group, and reduced the decrease in AlCl₃ groups. Nano-curcumin showed best antioxidant activity than curcumin in healthy and AlCl₃ groups. These results agrees with Bihaqi et al. [42] who’s reported that the treatment with AlCl₃ cause a significant decrease in of GST and GPX activities, meanwhile SOD activity was increased in compared with control. Also, AlCl₃ treatment caused significant decrease in the GSH concentration in plasma. Aluminum has been found to increase peroxidative damage to lipids, proteins and decreases antioxidant enzyme status [43]. On the other side, curcumin increased GSH content and decreased the level of lipid hydroperoxide in liver of rats injured by CCl4 [44]. Curcumin has been found to transact with oxidative stress in models of diabetes mellitus by increasing the activity of antioxidant enzymes such as superoxide dismutase 1 (SOD1), paraoxonase-1, glutathione peroxidase and catalase, which are main enzymes for the antioxidant preservation [45]. Treatment with curcumin increased GSH content in lymphocytes [46]. Soetikno et al. [47] found that treatment with curcumin prevented the decrease in GPx activity and decreased the oxidative stress, and renal fibrosis by modifying the normal pathway Nrf2-Keap1 in rats under nephrectomy. Curcumin showed ability to neutralize chemical carcinogens such as peroxyl, hydroxyl radical, superoxide and nitric oxide radical constitutes [48]. Curcumin-Nano partials showed excellent pharmacokinetics profiles compared to curcumin after a single oral dose of 50 mg/kg in rats [49]. The mean half-life of curcumin-Nano partials were 4 h, while, the mean half-life of curcumin was 1 h [50]. Furthermore, Zhang et al. [51] found that curcumin nanoparticle markedly suppressed the oxidative stress in rats. Curcumin-NPs (20 mg/kg) significantly increased the activities of SOD, catalase and GPx, in rat compared with curcumin (150, 300 mg/kg).

![Figure 5. Effect of curcumin, nano curcumin on TBARS, antioxidant enzymes (SOD and GST) and GSH content in plasma of male rats treated with aluminum chloride.](image-url)
4. Conclusion

Previous studies have suggested that aluminum plays a vital role in raising the signs of oxidative and inflammatory stress, which causes a strike in the nervous balance within the body. The results of the present study demonstrate that treating AlCl₃-treated rats with Curcumin and Nano-curcumin as antioxidant agents showed positive effects due to their ability to attenuate the reactivity of reactive oxygen species and to enhance activities of the antioxidant enzymes such as SOD and GST moreover the concentration of GSH. The present data showed that Curcumin and Nano-curcumin could be used to reduce the toxicity of AlCl₃.

5. Recommendations

This study recommends the use of curcumin and curcumin nanoparticles in the production of functional foods because of their anti-toxic properties, especially due to aluminum, and also has an excellent antioxidant activity.

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