Green Synthesis, Characterization, and Biological Evaluation of Hydroxyl-Capped Tellurium Nanoparticles

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Abstract

In this study, we used a simple green method for preparing tellurium nanoparticles and mainly evaluated their toxicological effects. The nanoparticles were synthesized using lactose and characterized with different instrumentation methods. The in vitro and in vivo cytotoxicity of tellurium nanoparticles and its effect on lipid profile were also evaluated. Hydroxyl-capped tellurium nanoparticles were successfully fabricated by lactose. The results showed spherical tellurium nanoparticles with a mean size of 89 nm. The toxicological study showed that the tellurium nanoparticles did not exhibit any toxicity on the primary cells. The LD_{50} values for the nanoparticles were 327 and 295 mg/kg for oral and intraperitoneal administrations, respectively. Also, the results showed a significant reduction in liver enzymes at the 16, 24, and 40 mg/kg doses. Hematological parameters indicated no significant suppressive changes between the animals that were administered tellurium nanoparticles and the control group. In addition, the effects of tellurium nanoparticles on hypercholesterolemic risk factors in mice fed with cholesterol demonstrated the depletion of triglyceride, cholesterol, and low-density lipoprotein. This study showed that the toxicity of tellurium nanoparticles was lower than tellurium ions. Furthermore, tellurium nanoparticles decreased the cholesterol and triglyceride levels in the animal model.

Keywords: Tellurium; Nanoparticles; Green synthesis; Toxicity; Cholesterol.

Introduction

Biomedical use of metallic nanoparticles, including antibacterial, antilishmaniasis, anti-inflammatory antioxidative activity, anti-atherosclerotic, and immunomodulatory, is undergoing significant

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expansion. Also tellurium is applicable as a photothermal and photo dynamic, antioxidative activity and reactive oxygen species (ROS) therapy, ROS sensing, and bioimaging agent. However, increasing the range of types and applications of these novel materials necessitates more investigations on the toxicity and health effects of exposure to nanoparticles (NPs) [1–4]. Tellurium (Te) is not an essential biological trace element, which is why this NP is neglected in biology [5]. Te is a common substance in electronics, metallurgy, and fluorescent CdTe quantum dots [3, 5, 6]. In medicine, bactericidal activity, inhibition of cytokine production, cancer therapy by immunomodulatory properties, antitussive activity, induction of hair growth, and as a protection agent in Parkinson’s disease and Te compounds have been recognized. However, few studies have examined the biological and toxicological properties of Te NPs in vivo and in vitro [7-9].

Recently, various forms of Te NPs such as nanorods, spherical particles, stars, and nanotubes were synthesized by different biological and chemical methods [10-15]. Most frequently method for the preparation of inorganic NPs is treating metal salts with chemical-reducing agents like hydrazine, fructose, sodium borohydride, and citric juice [1, 10-15]. In the the green synthesis method different plant extracts or natural compounds has been used as an environmentally-friendly approach for the synthesis of different nanoparticles [16]. During past years, the ability of saccharides to reduce Au and Ag ions to nanoparticles has been reported [16-19]. The oxidation of the hydroxyl residues of the saccharides to carbonyl groups facilitates the reduction of metallic ions to elemental metallic form [20, 21]. Fructose and starch are used to reduce Te ions to Te nanoparticles and nanowires [22, 23]. However, no report has been published on the synthesis of Te NPs using different saccharides such as lactose. On the other hand, some studies reported the depletion effect of Te ions on the triglyceride content of mice [24,25]. But no reports have been published on different biological effects of Te NPs fabricated by carbohydrates in literature. This study is the first report on the in vivo and in vitro toxicity and anti-hyperlipidemia effects of hydroxyl-capped Te NPs fabricated by lactose as a natural reducing agent.

Materials and Methods

1- Preparation of Te NPs

Respecting to synthesize Te NPs, potassium tellurite was subjected to reduction with different saccharides, and lactose was chosen for this purpose. Te NPs were synthesized by adding 1 g of lactose (Merck, Germany) to 100 ml of potassium tellurite solution (400 µg/ml). Potassium tellurite (K₂TeO₃) was purchased from Sigma-Aldrich (United States). The mixtures were then kept in an autoclave at 15 psi pressure, 121 °C for 15 minutes. The liquid color changed from colorless to black, which indicated the reduction of Te⁴⁺ ions to metallic Te NPs [7, 8]. The generated NPs were collected with sequential centrifugation at 12,000 ×g for 15 min, followed by dispersion of the pellet in deionized water to eliminate the excess lactose. For all experiments, the nanoparticles were washed and resuspended in deionized water and stored at four °C.

2- Characterization of Te NPs

The Te NP analysis was performed using different techniques. The UV-Vis spectrum of the nanoparticles was monitored with a UV-Vis Double Beam PC Scanning spectrophotometer (Labomed Model UVD-2950), with a resolution of 1 nm in the range of 200–600 nm. The particle size distribution pattern was determined with the laser light-scattering method using a Malvern Zetasizer MS2000 (UK). For the study of the functional groups on the surface of the NPs, the infrared spectrum of the dried pellet of nanoparticles was analyzed with Fourier transform infrared (FTIR) spectroscopy (Bomen MB-154). In the aim to examine the sample by transmission electron microscopy (TEM), one drop of the Te NP solution was placed on a carbon-coated copper grid and dried slowly at room temperature. Micrographs were obtained by using a Zeiss 902A TEM operated at 80 kV. The energy dispersive X-ray (EDX) microanalyzer was used to determine the elemental composition of the Te NPs. The crystalline structure of the Te NPs was checked with the XRD (X-ray diffraction) technique using an X-ray diffractometer (Philips PW1710) with CuKα radiation (λ = 1.5405 Å) over a scanning range of Bragg angles from 20° to 80°.

3- MTT assay

Splenic and hepatocyte cells were isolated according to Crawford’s [28] and Fry’s [29] methods, respectively. RPMI medium was supplemented with 10% (v/v) FBS and 100 µg and 100 IU of antibiotics (Pen-Strep) and used for cell culture. Cells were cultured in mentioned medium and then incubated at 37 °C, 5% CO₂, and 95% humidity for 24 h. Cellular growth with and without Te NPs was tested by measuring the degree of mitochondrial function and reduction of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan crystals [26]. In the next step, we harvested the rapidly growing cells.

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Then, the concentration of cells was adjusted to $1 \times 10^6$ cells per ml and seeded into a 96-well plate with 100 μl in each well. All plates were incubated for 24 h. Thereafter, Te NPs were applied to the culture wells to reach the final concentrations of 0–100 μg/ml. After 24 h of incubation, the cells were treated with 20 μl of the MTT reagent at a 5 mg/ml concentration in PBS and incubated for a further four h. Consequently, the medium was discarded, and 150 μl DMSO was added to solubilize the formazan crystals. The optical densities were determined at 550 nm after 5 min shaking to mix the formazan in the solvent. An MTT assay was performed in triplicate.

4- Animals

Six- to eight-week-old male BALB/c mice, each weighing 25–30 g, were obtained from Tarbiat Modares University (Iran). The mice were housed in plastic cages at controlled temperature (22 ± 1 °C) and humidity (50 ± 10%) for a 12/12 light and dark cycle, and all mice were allowed free access to water and food and fed a standard mouse pellet diet. The experimental procedures were performed in accordance with the guidelines of the Tehran University of Medical Science (Iran).

5- Acute Toxicity

One hundred mice were randomly divided into 20 different groups, with five mice in each group. The animals in groups 1 to 18 were separately exposed to K$_2$TeO$_3$ and Te NPs diluted in 0.9% apyrogenic NaCl solution with different Te concentrations listed in Table 1 by a single or gavage or intraperitoneal (i.p.) administration. In similar conditions, groups 19 and 20 were the control and received 0.9% sterile NaCl orally or i.p. The clinical signs of toxicity were observed for 14 days. Regarding determining the median lethal dose (LD$_{50}$), the mortality in the first 24 h was used.

6- Evaluation of Subacute Toxicity and Evaluation of Hepatic Enzymes and Hematological Parameters

To evaluate the subacute toxicity, we measured hepatic enzymes and hematological parameters. For this purpose, 30 mice were prepared for each test. The mice were randomly divided into five groups, four Te NPs-treated mice groups and one control group. For subacute profiling, four different doses of Te NPs suspended in saline solution (8, 16, 24, and 40 mg/kg) were administered orally by gavage, once per day for 14 days. The control group received only normal saline. To evaluate the hepatic enzymes, we anesthetized animals involved in the subacute toxicity test after overnight fasting, using ketamine-xylazine. The blood samples were collected from the heart and then incubated at 25 °C for 10 min so clotting would occur. The serum was separated from the blood clot by centrifugation at 2,500 ×g for 20 min at four °C. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using Ellitech diagnostic kits (France). Hematological analysis was carried out on whole blood collected in EDTA-containing tubes. The biochemical parameters, including red blood cells (RBC) count, white blood cells (WBC) count, platelet count, hematocrit percentage, and hemoglobin level, were examined with a hematology analyzer (Technicon H1; Bayer Medical Systems, USA).

7- Evaluation of Antihyperlipidemic Effect

The experiment was carried out in accordance with the method described by Hasimun et al. [31]. Twenty-four Balb/c mice were divided into four groups. Three groups received 10 mg/kg propylthiouracil (PTU) orally by gavage once per day and 0.01% PTU in drinking water once per day. Group 4 was the control group and did not receive any treatment. After seven days, two drops of blood were drawn from the preorbital cavity to evaluate the initial cholesterol level with Cardio Check PA (USA). Then, groups 1 and 2 received Te NPs (16 mg/kg) and lovastatin (5 mg/kg; Abidi Pharmaceutical Co., Iran), respectively, and group 3 was the negative control and received only PTU. After one h, a single dose of cholesterol (400 mg/kg) in vegetable oil was gavaged to all groups except group 4. Three hours later, the animals were sacrificed to obtain blood samples from the heart. The serum was separated from the blood clot by centrifugation at 2,500 ×g for 20 min. The serum samples were used to assess the lipid profile with

<table>
<thead>
<tr>
<th>K$_2$TeO$_3$ Dose oral (mg kg$^{-1}$)</th>
<th>Mouse mortality (%)</th>
<th>IP Te NPs (mg kg$^{-1}$)</th>
<th>Mouse mortality (%)</th>
<th>Oral Te NPs (mg kg$^{-1}$)</th>
<th>Mouse mortality (%)</th>
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<tbody>
<tr>
<td>10</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>10</td>
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<tr>
<td>25</td>
<td>12.5</td>
<td>100</td>
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<td>100</td>
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<tr>
<td>40</td>
<td>33</td>
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<tr>
<td>60</td>
<td>67</td>
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<td>0</td>
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<tr>
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<td>600</td>
<td>67</td>
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</table>
commercial enzymatic kits.

8- Statistical Analysis
The data are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to evaluate the mean values of the treatment groups, followed by Dunnett’s test to compare each group with control.

Results and Discussion
1- Green Synthesis
The color of the reaction mixture of potassium tellurite and lactose was changed toward black after autoclavage, indicating the reduction of Te⁴⁺ ions into Te NPs [3,28]. The resulting NP solution maintained at room temperature was stable for two months without any signs of aggregation. The hydrogen bonds of saccharides in an aqueous environment provide stable surface protection to prevent the aggregation of particles [16, 21].

The UV-visible spectrum of the NPs illustrated in Figure 1 represents the formation of the Te NPs due to the surface plasmon resonance at 243 and 295 nm. According to Gautam and Rao [29], the absorption range of 250–350 nm is sensitive to particle size. The TEM image of the nanoparticles shown in Figure 2 demonstrates no aggregated Te NPs with spherical shapes. The size distribution of the NPs analyzed with the laser light-scattering method showed the highest frequency of NPs (87%) at 89 nm. The elemental composition of nanostructures with EDX microanalysis (Fig. 3) confirmed the presence of Te atoms and oxygen groups, which could be related to the hydroxyl groups.

Figure 1. The UV-visible spectrum of the green synthesized Te NPs by lactose.

Figure 2. Transmission electron micrograph of Te NPs.

The peaks associated with Cu and C arise from the copper grid and carbon film used to prepare the samples. The phase and purity of the NPs were further
examined with X-ray diffraction (XRD, Fig. 4). The reflections in Figure 4 show the presence of broad peaks without any clear lattice parameters and an amorphous structure. FTIR spectroscopy was performed to determine the functional groups responsible for capping and stabilizing the Te NPs. Three bands at 1642.23, 2915.36, and 3444.91 cm\(^{-1}\) are ascribed to the carbonyl (C=O), hydrocarbon (C-H), and hydroxyl (O-H) stretching vibrations, respectively (Fig. 5). Based on these findings, –CO, –CH, and –OH groups were present on the surface of the nanoparticles. According to Qi et al. [30], these groups are saccharides distinctive which can bind to the surface of the NPs. The remarkable stability of NPs prepared using lactose could be attributed to the binding of hydroxyl groups to the NPs [30,31].

**In vitro Cytotoxicity of Te Nanoparticles using MTT Assay Method**

To evaluate the cytotoxicity of Te NPs on normal spleen and liver cells, we separately incubated subconfluent cultures of cells with different concentrations (20–100 µg/ml) of Te NPs for 24 h. Then the corresponding percentages of cell viability were calculated. The Te NPs’ effect on splenic and hepatocyte cells is depicted in Figure 6. Splenic cells’ cell viability was significantly increased at all concentrations of Te NPs compared to the control cells. The increased cell viability of liver cells was significant in all concentrations except at 20 µg/ml, which showed a slight increase up to 18%. The cytotoxicity of organic and inorganic Te ions has been well established, but the cytotoxicity effects of Te NPs have not been

![Figure 4. XRD spectrum of Te NPs prepared by green synthesis method.](image)

![Figure 5. FTIR spectrum of Te NPs green synthesized by lactose.](image)
investigated. Roy and Hardej [32] reported a significant decrease in hippocampal astrocytes cell viability in the rat at a range of concentrations from 45.5 ng/ml to 47.4 µg/ml of tellurium tetrachloride (TeCl₄) and diphenyl ditelluride (DPDT). The effects of TeCl₄ and DPDT on normal colon human cells were investigated by Vij and Hardej [32]. A decrease in cell viability was observed in the 370 ng/ml to 189.7 µg/ml range, which was significant at 94.8 and 189.7 µg/ml. Oxidative stress is toxic due to essential changes in the membrane and cytoplasm. Tellurium causes the oxidation of intracellular glutathione GSH, which can increase oxidative stress [8,32-34]. The results in this study suggest that Te NPs might have no reaction with GSH, which can prevent cytotoxic effects on tested cells.

2- Acute Toxicity and LD₅₀ in-vivo

Regarding determining LD₅₀, the experimental animals were exposed to different levels of Te NPs and K₂TeO₃ (Table 1). Te ions had the lowest LD₅₀ of 42 mg/kg (corresponding to 83.5 mg/kg K₂TeO₃), while orally administered Te NPs had the highest LD₅₀ (384 mg/kg). Therefore, Te ions were the most toxic to the mice. Comparing the measured LD₅₀ for Te ions and Te NPs indicated that green synthesized nanoparticles are approximately 9.15-fold less toxic in mice. Intraperitoneal injection of TeNPs showed a lower LD₅₀ (197 mg/kg) than oral administration (384 mg/kg), which revealed that toxicity depends on the route of administration. Although there have been some reports on the toxicity of Te ions in mammals [28, 32-36], nothing is known about the toxicity of metalloid Te NPs. A similar work showed that the toxicity (LD₅₀) of another salt of Tellurium (diphenyl ditelluride) was 61.4 mg/kg for i.p. administration [33] and was higher than the toxicity observed for the i.p. administration of Te NPs in this study.

3- Evaluation of Hepatic Enzymes and Biochemical Parameters

Subacute doses were chosen based on the results of the acute toxicity test. No death occurred in the administrated doses. Treatment of mice with 8, 16, 24, and 40 mg/kg of Te NPs orally for 14 days reduced the serum levels of liver enzymes (ALT and AST), which was significant at the 16, 24, and 40 mg/kg doses (Fig. 7). The highest percentages of decrease in ALT and AST were 42% and 33% at the NP doses of 16 and 24 mg/kg, respectively. The elevated serum levels of liver enzymes have been attributed to liver damage [37].
Therefore, these data suggest that Te NPs may have a hepatoprotective effect. The results of the hematological analysis are given in Table 2. No apparent changes were found at the tested doses of Te NPs compared to the control, showing no adverse effects on the biochemical parameters. However, the weight monitoring study results indicated that the bodyweight changes in animal test groups were fewer than the control group that no orally received Te NPs (Fig.8). We observed considerable digestive dysfunctions in the animals fed by Te NPs. This observation is critical, so further investigation needs to be performed to study possible side effects of Te NPs on the gastrointestinal system.

4- Antihyperlipidemic Effects
The cholesterol diet and PTU in the control group produced an almost 1.86-fold and 12.5-fold elevation in total cholesterol and triglyceride serum, respectively, compared to the sham group (Table 3). However, about a 4.5-fold and 1.06-fold increase in the very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) levels, respectively, were observed compared with the control group. The triglyceride levels and cholesterol were significantly depleted after the mice were treated with Te NPs compared with the control group. The depletion of the triglyceride levels was significant and showed approximately 80% attenuation. In addition, the

<table>
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<tr>
<th>Table 2. Blood hematology parameters in animals that orally received Te NPs against the control group</th>
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<tr>
<td><strong>Hematology parameters</strong></td>
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<tr>
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<tr>
<td>WBC (<em>×10^9/μL</em>)</td>
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<tr>
<td>RBC (<em>×10^12/μL</em>)</td>
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<tr>
<td>HGB (g/dL)</td>
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<tr>
<td>Hct (%)</td>
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<tr>
<td>M.C.V (fl)</td>
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<tr>
<td>MCH (pg)</td>
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<tr>
<td>M.C.H.C (g/dL)</td>
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<tr>
<td>PLT (<em>×10^3/μL</em>)</td>
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<tr>
<td>Neutrophils (%)</td>
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<tr>
<td>Lymphocyte (%)</td>
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<tr>
<td>Monocyte (%)</td>
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WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; Hct, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; PLT, platelet count.
*p<0.05, significantly different in comparison with control.
Te NP diet decreased the VLDL and LDL levels significantly, but no significant change in the HDL level was observed. Lovastatin showed a significantly changed lipid profile. The anti-elevating effect of Te NPs on cholesterol, TG, LDL, and HDL showed no significant difference compared with lovastatin, which is currently prescribed as an antihyperlipidemic agent. Te ions are known to block squalene oxidase, an enzyme involved in cholesterol biosynthesis. Squalene monooxygenase catalyzes the conversion of squalene to 2,3-oxidosqualene in the downstream pathway for cholesterol biosynthesis [38, 39]. Tellurium compounds react with thiol groups on squalene monooxygenase, which can cause blockage in cholesterol synthesis [38-40]. The significant depletion in the triglyceride and cholesterol levels in the cerebrum, cerebellum, and brainstem of albino mice using tellurium salts has been investigated [40]. Previously, it was reported that Lactobacillus plantarum with deposited Te NPs significantly reduction of serum triglycerides and cholesterol in mice fed PTU [28] and the beneficial effects of garlic on the cholesterol, triglyceride, LDL, and HDL of rats fed high cholesterol. Interestingly, garlic bulbs (Allium sativum) contain Tellurium, and its anti-hypercholesterolemia may contribute to the tellurium ions present in this plant’s highly beneficial bulbs [40].

**Conclusion**

This work showed the green synthesis of Te NPs and evaluation of NP toxicity conducted in vitro and in vivo models. Te NPs were successfully synthesized by using lactose as a reducing and stabilizing agent. Size distribution analysis showed the highest frequency of

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**Figure 8.** Bodyweight changes (mean) of animals that received different doses of Te NPs (8, 16, 24, and 40 mg/kg) for 14 days.

**Table 3.** Profile of lipids levels in serum of tested animals

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Sham group</th>
<th>Control group</th>
<th>Levostatin (5mg/kg)</th>
<th>Te NPs (16mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>100 ± 4.4</td>
<td>186 ± 9.5</td>
<td>158.7 ± 12.5*</td>
<td>148 ± 10.4*</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>50 ± 3</td>
<td>625 ± 83.2</td>
<td>93.2 ± 4*</td>
<td>121 ± 5.7*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>50 ± 2.6</td>
<td>74.75 ± 2.7</td>
<td>74.75 ± 2.7</td>
<td>77.25 ± 8</td>
</tr>
<tr>
<td>Cholesterol/HDL</td>
<td>2 ± 0.14</td>
<td>2.75 ± 0.4</td>
<td>2.1 ± 0.15*</td>
<td>1.9 ± 0.096*</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>8.2 ± 0.18</td>
<td>37 ± 2.45</td>
<td>18.75 ± 1</td>
<td>24.5 ± 4.9*</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>32.5 ± 1.3</td>
<td>34.5 ± 0.58</td>
<td>23.5 ± 2.38*</td>
<td>21.25 ± 1.7*</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>0.64 ± 0.03</td>
<td>0.5 ± 0.05</td>
<td>0.3 ± 0.05*</td>
<td>0.27 ± 0.05*</td>
</tr>
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</table>

Triglyceride; HDL, High-density lipoprotein; ch. Chylomicron; VLDL, Very low-density lipoprotein; LDL, low-density lipoprotein.

*p* < 0.05, significantly different when compared with control.
NPs at 89 nm. The Te NPs solution was stable for two months at room temperature. Aggregation reduces the nanoparticle’s surface area. So stabilizing of NPs and reducing their aggregation are essential properties for their specific activities. The hydroxyl groups of the saccharides provided an extensive network of hydrogen bonds, which act as a protective agent against the aggregation of nanoparticles. Carbonyl and hydroxyl groups can impoverish particles to engage in hydrogenic aggregation of nanoparticles. Carbonyl and hydroxyl bonds, which act as a protective agent against the reducing enzymes like GSH, CAT, and SOD which have antioxidant duties. Furthermore, Te NPs reduced the cholesterol and triglyceride levels in the animal model [28, 35, 44–45].

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