Research Article

Aziz Ullah Khan#, Mohamed Mohany#, Hidayat Ullah Khan*,#, Fozia Fozia#, Shahnaz Khan#, Naveed Kamran#, Fahim Ullah Khan#, Salim S. Al-Rejaie#, Ijaz Ahmad#, Nour S. S. Zaghloul#, Mourad A. M. Aboul-Soud*

Anti-Alzheimer, antioxidants, glucose-6-phosphate dehydrogenase effects of Taverniera glabra mediated ZnO and Fe2O3 nanoparticles in alloxan-induced diabetic rats

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Abstract: The current study aimed to assess the neuroprotective effect of Zn and Fe oxide nanoparticles biofabricated by Taverniera glabra in alloxan-induced diabetic rats. The experimental animals (160–200 g) were divided into nine groups (n = 9). The blood glucose, body weight, glucose-6-phosphate dehydrogenase (G6PD), superoxide dismutase (SOD), catalase test (CAT), lipid peroxidation (TBARS), glutathione (GSH), and acetycholinesterase (AChE) activities were determined. Oral administration of nanoparticles and T. glabra methanol extract (TGME; 10 and 15 mg/kg b.w) significantly decreased the glucose level, increased the body weight, controlled the quantitative level of G6PD, and significantly decreased the levels of ALT, ALP, cholesterol, and creatinine. Moreover, TGME and their Zn and Fe oxide nanoparticles significantly restored the antioxidant enzymes (SOD, CAT, GPx, and GSH) that decreased during induced diabetes. In the diabetic group, a significant increase in TBARS was noted and recovered in diabetic animals (p < 0.05) as compared to glibenclamide. The AChE activity was significantly recovered by nanoparticles and TGME both in the blood and brain of the diabetic group (p < 0.05). Taken together, it can be suggested that TGME and Zn and Fe oxide nanoparticles significantly improved memory and could be considered as an effective biogenic nanomaterial for diabetes, Alzheimer’s disease, and oxidative stress.

Keywords: diabetes, T. glabra, ZnO, Fe2O3NPs, oxidative stress, acetycholinesterase activities

1 Introduction

Diabetes mellitus is a chronic metabolic ailment categorized by improper secretion or function of insulin, resulting in increased blood glucose levels, and several other factors such as carbohydrates, proteins, and lipids that affect metabolism and function. It is also related to hyperglycemia,
which leads to oxidative stress [1]. According to the International Diabetes Federation report, the number of diabetic patients increased to above 592 million in the subsequent 25 years. Unlikely, 47% of the population is untreated and goes toward diabetic diseases ignorantly [2].

Blood contains the enzyme known as glucose-6-phosphate dehydrogenase (G6PD). Their deficiency is most frequently associated with the hexose monophosphate pathway disorder of red blood cells (RBCs) in humans. Approximately, this X-linked enzyme deficiency influences around 0.4 billion population globally [3]. In Pakistan, the occurrence of G6PD deficiency was found at about 1.8%. Various tribes of Pathans have shown a high frequency of G6PD deficiency (3.17%). Prevalence of G6PD deficiency is in Sindhis 2.77%, Punjabis 1.47%, and Kashmiris 1.07% [4]. Various extended and chronic diabetic complications, particularly in type 2 diabetes, are caused by reactive oxygen species (ROS) and an increase in free radicals. This compromises the body’s defense against antioxidant enzymes, damages cellular organelles and enzymes, and leads to problems [5]. Memory loss, neurological problems, instability in behavior, learning, planning, and daily living tasks are all symptoms of Alzheimer’s disease (AD) [6].

Neurofibrillary tangles (NFTs), senile plaques, and neuronal loss are the three major pathogenic features of AD brains [7]. A growing body of research has revealed that, in addition to the pathophysiology of senile plaques and NFT, widespread oxidative stress is a feature of AD brains. Superoxide dismutase (SOD) and catalase (CAT) are two examples of antioxidant enzymes whose activity or expression has been found to change in AD patients’ peripheral tissues as well as their central nervous system [8]. Furthermore, in AD and mild cognitive impairment brains, the enhanced oxidative loss of proteins and lipids and the decrease in glutathione and antioxidant enzyme potentials are confined to the synapses and associated with the cruelty of illness, indicating an intervention of oxidative stress in AD-related synaptic damage [9]. aberrant levels of butyrylcholinesterase (BChE) and acetyl-cholinesterase (AChE) are present in both type 2 diabetes mellitus (T2DM) and AD. AChE and BuChE levels that are altered in both AD and T2DM indicate that both of these enzymes may be crucial in the development of the two diseases. Elevated plasma concentrations of AChE and BuChE have shared characteristics with AD and T2DM [10]. Similarly, it has been discovered that diabetes patients’ AChE and BuChE activities are higher than those of normal controls [11]. This suggests that in addition to functioning as therapeutic targets, aberrant serum contents of AChE and BuChE may also serve as markers to anticipate the onset of T2DM and AD [12]. In the present study, we focused on the antidiabetic, and antioxidant effect of Zn and Fe oxide nanoparticles biofabricated by Taverniera glabra in alloxan-induced diabetic rats and to explore the beneficial effect of biofabricated nanoparticles in ameliorating neurodegeneration accompanied diabetes.

2 Materials and methods

2.1 Plant collection and authentication

A mature and fully grown-up plant of T. glabra was collected from the bank of Kurram River at Mardi Khel and Narmali Khel of District, Bannu KPK, Pakistan, while the plant Hypococum pendulum was collected from Karak and Bannu KPK, Pakistan. The taxonomical clarification and authentication were done by Professor A. Rehman, Dept. of Taxonomy, Govt Post Graduate College Bannu, Pakistan.

2.2 Plant extract collection

After identification, washed three times with tape water and then with distilled water. After washing, shade dried for several days, and then changed into fine power by a grinder. About 2 g of ground power was heated at 40°C in a 150 mL flask for 25 min with 60 mL of distilled water, and then cooled and filtered by Whatman filter paper No. 42. The filtrate was collected and stored at 4°C for further study.

2.3 Green synthesis of nanoparticles

FeCl₃·6H₂O solution of 1 mM for Fe₂O₃ NPs and 3 mM [Zn(CH₃COO)₂·2H₂O] solution for ZnO NPs were used as the precursor salts. By using a standard protocol, 10 mL of T. glabra extracts were put dropwise to 90 mL of respective salt solutions at pH 10 and 12.0, respectively. The mixture for Fe₂O₃ NPs was placed in a hotplate (60–80°C) and stirred for 8 h, while the mixture for ZnO NPs was stirred overnight. The synthesized nanoparticles were confirmed by various characterization techniques as previously published by us in Inorganic Chemistry Communications, 2022 (https://doi.org/10.1016/j.inoche.2022.110297).

2.4 Chemicals and reagents

During biological assays, analytical-grade substances such as glucometer, alloxan, Ellman’s reagent, trisodium citrate buffer,
vitamin C, ethylenediaminetetraacetate, 5-methylphenazinium methyl sulfate, and methanol were utilized, which were procured from Sigma-Aldrich, Germany.

2.5 Experimental animals

This study was conducted at the University of Science and Technology Bannu, Pakistan (Ethical committee Ref. no. ustb/biotech/70). Male Wistar albino rats (160–200 g b.w.) were obtained from the animal house, National University of Science and Technology, Pakistan (NUST), and placed in cages under standard environmental conditions (25 ± 1°C; 12 h light/dark cycle). The experimental rats were fed with standard pellet food and water obtained from the NUST, Pakistan. Before starting the experiment, the rats were left for 10 days so as to adjust to the new environment.

2.6 Toxicity assay of the rats

Toxicity assay of methanolic extract of T. glabra, and their mediated ZnO NPs and Fe₂O₃ NPs were done against two testing and one standard animal group. The rats fasted overnight, and then different doses (15 and 30 mg/kg b.w.) of extract and NPs were orally given to the experimental groups to evaluate their toxicity. Mortality and behavioral study of the experimental animals were observed for 24 h [13] and did not observe any acute toxicity, which showed that LD50 of the NPs is greater than 30 mg/kg. Based on this experiment, further study was performed using 10 and 15 mg/kg b.w. of methanolic extract of T. glabra, and their green synthesized ZnO NPs and Fe₂O₃ NPs.

2.7 Diabetes induction

The diabetic condition was developed in the investigational rats with a single intraperitoneal dose of alloxan monohydrate (120 mg/kg). After 72 h of alloxan injection, blood glucose levels were determined by a glucometer. During this period 5% glucose was given instead of water to decrease death due to hyperglycemia. Rats with glucose levels higher than 200 mg/dL were selected for the experiment [14].

2.8 Experimental design

After induction of diabetes, the animals were divided into nine groups (five animals in a group) Group I: normal control, Group II: untreated diabetic rats, Group III: administrated with glibenclamide, Groups IV and V: administrated with T. glabra methanol extract 10–15 mg/kg, Groups VI and VII were treated with ZnO NPs 10, 15 mg/kg, and Groups VIII and IX administrated with Fe₂O₃ NPs 10, 15 mg/kg 21 days orally feeding of the glibenclamide and test samples were given every morning by 16 gauge gastric intubation [15].

2.9 In vivo assessment

2.9.1 Blood sampling and body weight examination

Tested the fasting blood glucose levels at the beginning of experimental dose administration (t = 0; first day), subsequently on days 7, 14, and 21 of the dose treatments using CGMS glucometer (US). Blood samples were collected from their tail veins. The rats were weighted regularly from day first to last (first, seventh, fourteenth, and twenty-first) [14].

2.9.2 Biochemical analysis

After 21 days of treatment, the blood samples were collected on twenty-second day, stored in EDTA tubes and centrifuged at 3,000 rpm for 10 min and the collected serum was stored at −80°C for further analysis. Alanine transaminase (ALT), alkaline phosphatase (ALP), total cholesterol, and creatinine concentrations were examined by a chemistry analyzer (Selectra, XL, Netherlands) with commercially available kits (Gesan productions, Italy) according to the manufacturer’s protocols [16].

2.9.3 AChE potential in brain and blood

AChE potential of the brain and blood were analyzed with Ellman protocol with little modifications. The reaction mixture consisted of 7.5 mL K₃PO₄ buffer (260 mM, pH 7.5) and 0.001 M DTNB. For starting the reactions 800 μM acetylcholine iodide was added to 100 μL of brain and serum samples. The reaction mixtures were incubated for 15 min at 25°C. Then AchE efficacy was measured at an absorbance of 412 nm. Blood serum activity was calculated from the ratio between hemoglobin level and AChE potential, and measured in μmol AcSCh/h/mg of protein [17].

2.9.4 SOD potential

SOD assays were performed by the protocol of Yadav and Yadav [18]. SOD assays were started by mixing 150 μL of
blood samples, 650 µL of tetra-sodium phosphate, 0.05 µL of 5-methylphenazinium methyl sulfate, and 100 µL of reduced nicotinamide adenine dinucleotide (NAD + H). Then after 2 min, 500 µL of anhydrous acetic acid was added to break the reaction. Color strengths were calculated at 570 nm, and measured in the unit of E/min/mg protein.

2.9.5 CAT potential

CAT analysis was performed and initiated in graduated beakers by mixing 0.1 mL of blood serum samples and 0.15 mL of K3PO4 buffer (10 mM, pH 7), and then adding 0.25 mL of 160 mM hydrogen peroxide to commence the reactions. Then incubated at 97.6°F for 2 min. To break the reaction 1,000 µL of dichromate/acetic acid reagent was added. Boiled the mixture for 20 min, which changed the reaction 1,000 µL of dichromate/acetic acid reagent was added. Boiled the mixture for 20 min, which changed the reaction 1,000 µL of dichromate/acetic acid reagent was added. Boiled the mixture for 20 min, which changed the reaction 1,000 µL of dichromate/acetic acid reagent was added. Boiled the mixture for 20 min, which changes to green color. Color strengths were calculated at 570 nm, and measured in the unit of E/min/mg protein [14].

2.9.6 Glutathione peroxidase (GSP-Px) potential

GSP-Px potentials were performed by standard Ellman’s Reagent protocol [14]. Activities were performed by combining 1,490 µL Na3PO4 buffer (400 µM, pH 7.4), 100 µL ethylenediaminetetraaceticacid (400 µM), 100 µL sodium azide (0.005 M), and 50 µL glutathione (0.004 M) mixtures were arranged. Additionally, 0.1 mL of blood samples was added to the mixture, and were incubated at 98°F for 7 min. Then 100 µL of hydrogen peroxide (0.004 M) was added to the solution and measured the glutathione at 412 nm by spectrophotometer.

2.9.7 Glutathione-S-transferase (GST) potential

GST efficacies consisted of 20 µL 0.001 M dinitrochlorobenzene [C6H3Cl(NO2)2], 2,900 µL glutathione mixture, and 0.03 mL blood samples. The glutathione mixture was organized by mixing 300 µg of glutathione in 200 mM of pH 7.5 M PO4 buffer. Changes in the yellow color of the activities were measured at 340 nm by spectrophotometer in units of GSH/mg/protein [14].

2.9.8 Lipid peroxidation assay (TBARS)

Iqbal et al. analyzed thiobarbituric acid reactive substance (TBARS) levels in lipid peroxidation assays [19]. The reaction mixture contained 100 mM pH 7.5 K3PO4 buffer (580 µL) having 200 µL blood samples, 200 µL vitamin C, and 20 µL FeCl3. The mixture was incubated at 98.6°F for 55 min and regularly shacked in a water bath. To break up the reaction 10% CCl3COOH was added. Afterward heated for 25 min and then cooled in an ice bath. After cooling centrifuged at room temperature for 12 min. Mixtures were scanned for absorbance at 535 nm wavelength.

2.9.9 Effect of G6PD in animal models

The conversion of a dye color (color changes from blue to brown) takes 30–60 min. Dye color conversion (color changes from blue to brown) takes 2–24 h.

2.9.10 Quantitative test of G6PD

The quantitative test (UV-Kinetic Method) for G6PD deficiency was accomplished by a commercially existing kit (ENZOPAK, Recon Diagnostic Pvt. Ltd). The three reagents in the kit were G6PDH-coenzyme substrate, G6PDH-buffer, G6PDH-lysing reagent. G6PD deficiency tests were performed from fresh heparinized blood.

G6PDH activity (at 30°C) = 146–376 U/1,012 RBC or 4.6–13.5 U/g Hb [20].

2.9.11 Statistical study

All data were expressed as the mean ± standard error (SEM). Statistical significance was determined using one-way analysis of variance, followed by post-hoc multiple comparisons (Tukey test) using GraphPad Prism version 8 (GraphPad Software, Inc., La Jolla, CA, USA). p ≤ 0.05 was considered to indicate a statistically significant difference.

3 Results

3.1 Acute toxicity test

A single dose of methanolic extract of T. glabra, and their mediated ZnO NPs and Fe2O3 NPs, have shown no mortality or any physical acute toxicity symptoms in treated animals during the entire experimental period. Acute toxicity assessment has presented that methanolic extract of T. glabra, and their mediated ZnO NPs and Fe2O3 NPs is
safe up to 30 mg/kg body weight. So 10 mg/kg and 15 mg/kg b.w. dosages were static as adequate concentrations, and thus used in the alloxan-induced diabetic rat’s activity.

### 3.2 Effect of methanolic extract, and their mediated ZnO NPs and Fe₂O₃ NPs on blood glucose and body weight

Tables 1 and 2 illustrate blood glucose level and body weight of G6PD deficient non-diabetic control, diabetic control, methanolic extract of *T. glabra*-treated diabetic rats, and their mediated ZnO NPs and Fe₂O₃ NPs treated diabetic rats at first, seventh, fourteenth, and twenty-first, respectively. The diabetic group had considerably lower body weights (128.04 ± 2.74 vs 181.49 ± 1.08) and higher blood glucose levels as compared to the non-diabetic control group (294.76 ± 5.07 vs 91.56 ± 1.36). By oral administration of methanolic extract of *T. glabra*, and their mediated ZnO NPs and Fe₂O₃ NPs which in turn normalized blood glucose levels (Table 1) and body weight (Table 2) in the treated diabetic groups compared with non-treated diabetic groups. In every group, however, there was a considerable decrease in blood glucose level (Table 1) and an increase in the body weight (Table 2) occurred due to G6PD regulation that we experienced in samples treated diabetic rats compared with the diabetic rats.

### 3.3 Effect of *T. glabra* methanolic extract, and their mediated ZnO NPs and Fe₂O₃ NPs on biochemical parameters

When diabetic rats were compared to non-diabetic controls, the G6PD level was significantly decreased (*p* < 0.05) (Table 3). As G6PD levels decreased, diabetic rats

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**Table 1: Effect of *T. glabra* methanolic extract, and their mediated ZnO NPs and Fe₂O₃ NPs on blood glucose in experimental animals**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Blood glucose level (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
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<tr>
<td>Normal control</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide (0.5 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + METg (10 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + METg (15 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + ZnO NPs (10 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + ZnO NPs (15 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + FeO NPs (10 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + FeO NPs (15 mg/kg)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The data (n = 5) showing as mean ± SEM, showing significance at *p* < 0.05: normal control vs diabetic control; #p < 0.05: diabetic control vs treated groups. METg: methanolic extract of *T. glabra*.

**Table 2: Effect of *T. glabra* methanolic extract, and their mediated ZnO NPs and Fe₂O₃ NPs on body weight of experimental animals**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Body weight variation (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal control</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide (0.5 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + METg (10 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + METg (15 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + ZnO NPs (10 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + ZnO NPs (15 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + FeO NPs (10 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Diabetic + FeO NPs (15 mg/kg)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The data (n = 5) showing as mean ± SEM, showing significance at *p* < 0.05: normal control vs diabetic control; #p < 0.05: diabetic control vs treated groups. METg: methanolic extract of *T. glabra*.
Table 3: Effect of T. glabra methanolic extract, and their mediated ZnO NPs and Fe2O3 NPs on G6PD, ALT, ALP, creatinine, and cholesterol on diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G6PD (min)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Creatinine (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>25.63 ± 0.56</td>
<td>32 ± 2.9</td>
<td>118 ± 2.9</td>
<td>0.54 ± 0.08</td>
<td>39.65 ± 5.8</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>&gt;9*</td>
<td>86 ± 5.3*</td>
<td>263 ± 5.7*</td>
<td>1.78 ± 0.11*</td>
<td>107.64 ± 5.7*</td>
</tr>
<tr>
<td>Glibenclamide (0.5 mg/kg)</td>
<td>31.26 ± 0.52</td>
<td>39 ± 4.5</td>
<td>126 ± 4.6</td>
<td>0.62 ± 0.07</td>
<td>45.29 ± 4.9</td>
</tr>
<tr>
<td>Diabetic + METg (10 mg/kg)</td>
<td>49.33 ± 0.76</td>
<td>68 ± 5.8*</td>
<td>173 ± 4.8*</td>
<td>1.09 ± 0.05*</td>
<td>91.64 ± 5.3*</td>
</tr>
<tr>
<td>Diabetic + METg (15 mg/kg)</td>
<td>44.05 ± 0.35</td>
<td>45 ± 4.6*</td>
<td>140 ± 5.3*</td>
<td>0.81 ± 0.04*</td>
<td>59.21 ± 5.1*</td>
</tr>
<tr>
<td>Diabetic + ZnO NPs (10 mg/kg)</td>
<td>39.19 ± 0.28</td>
<td>61 ± 3.5*</td>
<td>162 ± 5.7*</td>
<td>1.04 ± 0.05*</td>
<td>86.02 ± 4.9*</td>
</tr>
<tr>
<td>Diabetic + ZnO NPs (15 mg/kg)</td>
<td>30.42 ± 0.40</td>
<td>42 ± 5.8*</td>
<td>134 ± 4.5*</td>
<td>0.79 ± 0.03*</td>
<td>53.07 ± 5.2*</td>
</tr>
<tr>
<td>Diabetic + Fe2O3 NPs (10 mg/kg)</td>
<td>53.83 ± 1.06</td>
<td>65 ± 5.7*</td>
<td>179 ± 5.8*</td>
<td>1.25 ± 0.14*</td>
<td>92.66 ± 5.8*</td>
</tr>
<tr>
<td>Diabetic + Fe2O3 NPs (15 mg/kg)</td>
<td>48.35 ± 1.15</td>
<td>48 ± 6.2*</td>
<td>145 ± 5.9*</td>
<td>0.86 ± 0.09*</td>
<td>59.93 ± 5.4*</td>
</tr>
</tbody>
</table>

Note: The data (n = 5) showing as mean ± SEM, showing significance at *p < 0.05: normal control vs diabetic control; #p < 0.05: diabetic control vs treated groups. METg: methanolic extract of T. glabra.

also had significantly (p < 0.05) higher levels of ALT (86 ± 5.3 IU/L vs 32 ± 2.9 IU/L), ALP (263 ± 5.7 IU/L vs 118 ± 2.9 IU/L), cholesterol (107.64 ± 5.7 mg/dL vs 39.65 ± 5.8 mg/dL), and creatinine (1.78 0.11 mg/dL vs 0.54 ± 0.08 mg/dL). The lack of G6PD enzyme in diabetic rats was sustained by glibenclamide, a methanolic extract of T. glabra, and its mediated ZnO NPs and Fe2O3 NPs treatments. The change in ALT, ALP, cholesterol, and creatinine (Table 3) in diabetic rats likewise returned to normal as the G6PD deficit stabilized. It was hypothesized that nanoparticles and T. glabra methanolic extract administration could regulate blood parameters and stabilize the G6PD enzyme as a result of the significant reduction (p < 0.05) in G6PD level in diabetic rats (Table 3).

3.4 Effect of methanolic extract of T. glabra, and their mediated ZnO NPs and Fe2O3 NPs on antioxidant enzymes

Results found that the concentrations of antioxidant enzymes such as SOD, CAT, GPx, and GSH were significantly decreased, while TBARS concentration was increased in diabetic rats as compared to non-diabetic rats (Figure 1). In G6PD deficient, a significant decrease (p < 0.05) in SOD (8.36 ± 0.91 vs 8.36 ± 0.91) SOD/mg of protein, CAT (3.37 ± 2.8 vs 7.45 ± 3.2) E/min/mg of protein, GPx (3.79 ± 1.3 vs 9.01 ± 3.5) nmol/NADPH/min/mg of protein, and GSH (44.56 ± 5.9 vs 98.74 ± 7.6) GSH/mg of protein were examined in diabetic rats, while the increase in TBARS level (3.67 ± 0.11 vs 1.84 ± 0.08) nmol MDA/mg of protein were observed. The deficits in antioxidant indicators, such as SOD, CAT, GPx, GSH, and TBARS elevations, as shown in Figure 1, were corrected by oral administration of T. glabra extract and their mediated zinc and iron oxide nanoparticles (10 and 15 mg/kg).

3.5 Effect of methanolic extract of T. glabra, and their mediated ZnO NPs and Fe2O3 NPs on AChE potential in different brain portions and blood of diabetic rats

Results showed that the AChE level in the homogenate of the brain was significantly (p < 0.05) higher in diabetic rats as compared to normal healthy rats. The AChE potential was significantly (p < 0.05) reduced in the rats which were given methanolic extracts of T. glabra and their mediated zinc and iron oxide nanoparticles (10 and 15 mg/kg body weight, respectively) in the cerebral cortex, cerebellum, hypothalamus, striatum, and hippocampus (Table 4). Additionally, it was discovered that diabetic rats’ blood levels of the AChE enzyme were significantly higher (p < 0.05) than those of normal healthy rats. At the end of the trial (21 days), the treatment of methanolic extracts of T. glabra, ZnO NPs, and Fe2O3 NPs (10 and 15 mg/kg b.w.) considerably reduced the increased concentrations of AChE in the blood of diabetic rats (Figure 2).

4 Discussion

In the current study, we show that alloxan-induced diabetic rats had hyperglycemia, lower body weights, elevated liver functions, creatinine, cholesterol, and oxidative stress, as well as lower levels of G6PD and antioxidant enzymes and significantly higher AChE activity in the
blood and various brain regions. The pathophysiology of diabetic neuropathy is one that is rapidly expanding and should be of worldwide concern. In most situations, diabetic neuropathy begins as a modest, serum glucose-dependent fiber neuropathy. Discovering novel substances and treatment approaches to reduce the hazards associated with diabetes is of great interest. As a result, in the current study, we investigated the neuroprotective and ameliorative potential of Zn and Fe oxide nanoparticles biofabricated by *T. glabra* in rats with alloxan-induced diabetes. The acute toxicity assay did not show any toxic behavior of methanol extract and biochemically synthesized ZnO NPs and Fe$_2$O$_3$ NPs. The alloxan-induced diabetic rats administered with different concentrations of *T. glabra* methanolic extract, and their mediated ZnO NPs and Fe$_2$O$_3$ NPs, have not exposed any physical mark of harmfulness in the entire experiment, and no deaths were observed. Based on this study, with different doses of methanol extract, ZnO

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![Figure 1](image_url): Effect of *T. glabra* methanolic extract and their mediated ZnO NPs and Fe$_2$O$_3$ NPs on the TBARS and SOD, CAT, GPx, and GSH enzyme activities in diabetic rats. *p < 0.05: normal control vs diabetic control; #p < 0.05: diabetic control vs treated groups. METg: methanolic extract of *T. glabra*. 

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NPs and Fe$_2$O$_3$ NPs, 10 and 15 mg/kg body weights were taken as a suitable dose, and so used for administration of alloxan-treated animals.

The blood glucose levels have been observed in the whole treatment, to assess the effect on G6PD and the anti-diabetic effect of methanolic extract and green synthesized nanoparticles (ZnO NPs, Fe$_2$O$_3$ NPs). At the end of the experiment (twenty-first day) the administrated groups (extract and NPs) noticeably stabilized G6PD and reduced the blood glucose concentration compared to diabetic control (Group II). It was reported that the activity of G6PD in animal studies was enhanced when treated with zinc, as it takes part in the activation of zinc enzymes [21]. ZnO NPs have revealed low blood glucose level compared to Fe$_2$O$_3$ NPs and plant extract. On the other hand, glibenclamide stabilized G6PD considerably and so decreased the blood glucose level in the whole period of administration compared to other groups.

Alloxan-induced diabetic animal studies have been generally used in numerous studies, and the way of action of alloxan is healthy and accepted [22,23]. In the treatment of diabetes, the blood glucose level is considered a main biochemical marker to observe the progress in the disease disorder [23]. Table 2 shows the variations in body weight of investigational rats, before and after dose administration. Body weight loss is the main sign related with diabetes, possibly due to muscle wasting [24]. In our study the alloxan-induced diabetic rats group (Group II) showed significant weight loss (Table 2) compared normal control (Group I). The ALT, ALP, creatinine, and total cholesterol levels were also high in diabetic rats (Table 3).

It was reported that diabetes mellitus decreases the body weight due to increased muscle deteriorating, dehydration, and fat catabolism. Whereas, G6PD treated by plant extract and nanoparticles significantly increased

Table 4: Effect of $T$. glabra methanolic extract and their mediated ZnO NPs and Fe$_2$O$_3$ NPs on AChE potential in different brain portions of diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cerebral cortex</th>
<th>Cerebellum</th>
<th>Hypothalamus</th>
<th>Striatum</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>54.7 ± 2.16</td>
<td>48.23 ± 2.34</td>
<td>44.8 ± 4.51</td>
<td>41.3 ± 3.7</td>
<td>40.53 ± 5.14</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>83.5 ± 5.23*</td>
<td>76.14 ± 3.43*</td>
<td>73.9 ± 5.43*</td>
<td>71.5 ± 4.5*</td>
<td>72.15 ± 5.12*</td>
</tr>
<tr>
<td>Glibenclamide (0.5 mg/kg)</td>
<td>57.9 ± 3.05</td>
<td>52.32 ± 2.37</td>
<td>47.5 ± 3.80</td>
<td>43.2 ± 3.4</td>
<td>43.27 ± 5.09</td>
</tr>
<tr>
<td>Diabetic + METg (10 mg/kg)</td>
<td>81.4 ± 5.21*</td>
<td>74.21 ± 3.51*</td>
<td>71.6 ± 5.54*</td>
<td>70.4 ± 5.5*</td>
<td>70.53 ± 7.10*</td>
</tr>
<tr>
<td>Diabetic + FE Tg (15 mg/kg)</td>
<td>68.5 ± 4.37*</td>
<td>63.42 ± 3.23*</td>
<td>61.2 ± 4.32*</td>
<td>59.5 ± 4.6*</td>
<td>58.45 ± 7.24*</td>
</tr>
<tr>
<td>Diabetic + ZnO NPs (10 mg/kg)</td>
<td>74.4 ± 5.12*</td>
<td>69.35 ± 4.13*</td>
<td>64.4 ± 5.03*</td>
<td>61.7 ± 4.3*</td>
<td>62.57 ± 5.43*</td>
</tr>
<tr>
<td>Diabetic + ZnO NPs (15 mg/kg)</td>
<td>61.9 ± 4.05*</td>
<td>54.26 ± 3.17*</td>
<td>48.5 ± 3.25*</td>
<td>46.9 ± 4.4*</td>
<td>45.62 ± 5.21*</td>
</tr>
<tr>
<td>Diabetic + FeO NPs (10 mg/kg)</td>
<td>76.5 ± 5.23*</td>
<td>71.63 ± 5.51*</td>
<td>68.3 ± 5.30*</td>
<td>66.5 ± 5.3*</td>
<td>68.33 ± 6.29*</td>
</tr>
<tr>
<td>Diabetic + FeO NPs (15 mg/kg)</td>
<td>62.3 ± 5.10*</td>
<td>58.52 ± 3.46*</td>
<td>56.1 ± 4.17*</td>
<td>53.4 ± 4.5*</td>
<td>51.07 ± 5.67*</td>
</tr>
</tbody>
</table>

Note: The data ($n = 5$) showing as mean ± SEM, showing significance at $p < 0.05$: normal control vs diabetic control; $p < 0.05$: diabetic control vs treated groups. METg: methanolic extract of $T$. glabra, measuring unit = µmol AcSCh/h/mg of protein.

Figure 2: Effect of $T$. glabra methanolic extract and their mediated ZnO NPs and Fe$_2$O$_3$ NPs on AChE activity in the blood of diabetic rats. The data ($n = 5$) showing as mean ± SEM, showing significance at $p < 0.05$: normal control vs diabetic control; $p < 0.05$: diabetic control vs treated groups. METg (methanolic extract of $T$. glabra).
the body weight loss. The ALT, ALP, creatinine, and total cholesterol levels were also recovered in diabetic rats over a period of 21 days treatment (Table 3). The mechanism of inhibiting the muscle loss could possibly attribute to the reverse of antagonism [25].

Research presented that elevated glucose levels leads to enhanced oxidative stress in diabetic rats and patients [26]. Too much evidence are present for increased oxidative stress in diabetic problems. Research has shown that cultured cells allowed high glucose to have high oxidative stress as found by higher levels of lipid peroxidation and high level of ROS [26,27]. Another researcher cultured cells and administrated to high levels of antioxidants (GSH, SOD, or CAT) are defensive against harmful effects of high glucose level. Similarly, ROS species have been presented that these may be either the cause or may be a concern with the path that have presented to facilitate diabetic disorders, such as aldose reductase activity showed exhaustion of NADPH, and as a result ROS species are increased [27]. Thus these evidence showed that high oxidative stress is the main cause of diabetic mellitus disorders.

Anti-oxidative system is preserved and maintained by adequate production of reductants like NADPH. As NADPH is the main cofactor of CAT enzyme as it retains the enzyme in active form. NADPH is also the cofactor of glutathione reductase, which is a free radical scavenger. As the main source of NADPH, the function of G6PD as an antioxidant enzyme has been currently healthily particularized. It was presented that G6PD-null cells have better sensitivity against oxidative stress [28]. Furthermore, G6PD supports the protection of oxidative stress-created apoptosis [29]. It was reported that elevated glucose levels in cultured endothelial cells inhibited the activity of G6PD [30]. D’Alessandro et al. presented that G6PD activity was greatly inhibited and decreased in both glomeruli and tubules of the kidney of diabetic mice compared with normal control [31]. Data from the current study proposed that G6PD activity of the liver and kidney in diabetic animals was inhibited through elevated blood glucose levels compared to normal control. This study is reliable with the previous available studies in which deficiency of G6PD activities have been reported in the liver, aorta, and heart cells from diabetic animal modules [31,32]. Diabetic patients possess reduced G6PD levels in the liver. Hemolysis of blood cells created G6PD deficiency in diabetes mellitus patients, particularly in newborns were also reported [33].

Systematic observations of G6PD activity of some people suggested an enhanced occurrence of G6PD deficiency in people with diabetes, as compared to normal people [34]. So many studies are performed about the association between G6PD deficiency and diabetes mellitus and has been a matter of debate. The hypothesis revealed that G6PD deficiency is related to hyperglycemia and diabetic conditions are increased [35]. In contrast to other studies, Salomon et al. revealed no notable changes in G6PD deficiency offspring compared to G6PD control, presenting the lack of diabetes in the newborns. In G6PD deficient newborns serum bilirubin, AST, and ALT levels were found higher than in G6PD control [36]. The hypothesis of liver malfunctions and damage is reported to have a high quantity of cholesterol and ALT in several G6PD deficient patients [37].

Diabetes mellitus is well-documented for its capacity to cause oxidative stress in a variety of tissues. A significant extent of superoxide and supplementary free radicals are released in the cytoplasm, because of the increased mitochondrial glucose oxidation brought on by hyperglycemia. Recent research stated that the production of advanced glycation end products, due to which superoxide formation takes place, is caused by chronic hyperglycemia. As a result, NADPH oxidase is activated, increasing the production of superoxide. The escalation of MDA concentrations while the decline in SOD and GSH activity of cells were indicators of an oxidative stress state brought on by diabetes [38].

The influence of methanolic extract, zinc oxide, and iron oxide nanoparticles on lipid peroxidation (MDA), SOD, CAT, GPx, and GSH, our findings presented that there is a significant decrease in the concentrations of SOD, CAT, GPx, and GSH, while an increase in the concentration of MDA in the blood of diabetic animals. In contrast to these methanolic extracts, zinc oxide and iron oxide nanoparticles increase SOD, CAT, GPx, and GSH, while decreasing the level of MDA, decreases an essential element in different antioxidant enzymes such as Zn-SOD, Zn-metallothionein, etc. [39]. Several researchers examined that Zn-metallothionein along with islet cells gives defense against various free radicles that originated in the body originating sources and causes. The cell’s potential to defend itself from this oxide itself uses as intracellular Zn levels get more depleted. This is a hypothetical mechanism by which a zinc deficiency shows how rapidly diabetes mellitus develops. Gupta et al. examined the lipid peroxide levels in the nervous system of animals nourished by nutrition low in zinc and discovered that SOD levels were decreased in the cerebrum, cerebellum, hypothalamus, hippocampus, brainstem, while peroxide levels increased in several areas of the brain and spinal cord [40].

According to Aruoma, Cu, Zn, and Mn are essential elements for the potentials of the respective SOD enzymes.
The present research project proposes that the *T. glabra* methanolic extract and their mediated ZnO NP and Fe₂O₃ NP have significant effect on oxidative stress and G6PD, which in turn regulate ALT, ALP, creatinine, and cholesterol levels in diabetic conditions. Finally, it is concluded that the extract of *T. glabra* methanolic extract and their mediated ZnO NP and Fe₂O₃ NP are valuable source of neuroprotective, antioxidative, and antidiabetic candidates with special reference to ameliorate one of the most complications of diabetes (diabetic neuropathy). Further analysis is required regarding its pharmacetical and therapeutic potentials.

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**Author contributions:** All authors participated in the design and interpretation of studies, the analysis of the data, and the review of the manuscript. Aziz Ullah Khan, Hidayat Ullah Khan, Fahim Ullah Khan, and Fozia Fozia conducted the experiments and collected the data, Shahnaz Khan, Naveed Kamran, and Ijaz Ahmad were responsible for the analysis and mapping of the data. Mohamed Mohany provided methodological and technical guidance. Mourad A.M. Aboul-Soud wrote the manuscript, and Nouf S.S. Zaghloul, Salim S. Al-Rejaie, and Mohamed Mohany project administration, edited, and reviewed the manuscript. All authors read and approved the final manuscript.

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**Ethical approval:** The research related to animals’ use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

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**References**


