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Green Synthesis of ZnO Nanoparticles using *Eryngium billardieri* Leaf Extract: Characterization and its Anti-Diabetic Properties

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Abstract

The present study developed the green synthesis approach to prepare ZnO nanoparticles (ZnONPs) with the aid of *Eryngium billardieri* leaf extract as an efficient stabilizer. The properties of the fabricated nanoparticles were determined by TEM, FE-SEM, XRD, EDX, FT-IR, TGA, and UV-vis DR spectra techniques. The results obtained from FE-SEM and TEM images displayed that the size of ZnONPs synthesized in the presence of the leaf extract in compared to the chemically fabricated sample was decreased. According to the results from UV-vis DRS spectra, EDX, FTIR, and TG analyses, presence of some bio-molecules from the plant extract on the surface of ZnONPs was confirmed. The green synthesis of nanoparticles using extracts and their potential applications as anti-diabetic agents has attracted a great attention in recent years. Anti-diabetic activity of the prepared sample was detected by treating alloxan-induced diabetic rats compared with those treated with the *E. billardieri* leaf extract and insulin. The pre and post treatments, levels of fasting blood sugar, total cholesterol, insulin, high-density lipoprotein, and total triglyceride were determined. Analysis in the diabetic rats displayed an increase in high-density lipoprotein and a significant decrease in fasting blood sugar levels in all groups under treatment. Furthermore, cholesterol reduction was observed in the group treated by ZnO powder prepared in the presence of the extract. The anti-diabetic results indicated that the green ZnONPs have an excellent efficiency in overcoming diabetic rats.

Keywords: Green synthesis; ZnONPs; Diabetes; Insulin; Lipid profile

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Introduction

Zinc is a crucial trace element for animal cells. It is a versatile material and second most common element in the human body, which shows a significant role in bioactivities like the hepatic glycogenesis and metabolism of glucose [1,2]. Zinc oxide (ZnO) has several applications such as optoelectronics, electronics, photocatalysts, sensors, detectors, and solar cells. This metal oxide is non-toxic, eco-friendly, and low-cost [3–6]. ZnONPs shows an antiviral, antibacterial, antifungal, anticancer, and anti-diabetic activity [7]. There are numerous chemical/physical approaches to fabricate ZnONPs such as sol-gel method, co-precipitation, spray pyrolysis, thermal decomposition, and hydrothermal synthesis [8–10]. These methods generally require high cost of operation, high temperature, and chemical solvents and toxic substances [11]. Microwave method is a facile, controllable, and fast to fabricate these nanostructures [12,13]. On the other hand, plant extracts and microorganisms are eco-friendly approaches for fabrication of the ZnONPs [14–18]. Green synthesis method, using plant extracts have been accepted as a promising route due to their biological nature, availability, and low cost [19–22].

Eryngium billardieri belongs to the umbliferae family that is an endemic plant from Asia and widely grows in several regions of Iran [23–25]. This plant contains alkaloids, flavonoids, triterpenes, acetylene, monoterpene, tannin, sucrose, caffeicacid, chlorogenic acid, saponins, and coumarone [26,27]. *E. billardieri* is a plant with anti-inflammatory, antioxidant, antimicrobial, anti-diabetic activities [28,29].

In the present paper, pure ZnONPs in water (ZnO) and fabricated

by the green technique in the extract (ZnO (E)) were investigated for treatment of 30 male Wistar rats (5 rats in 6 groups). In addition, the XRD, FE-SEM, EDX, TEM, FT-IR, TGA, and UV-vis DR spectra instruments were used for characterization of the fabricated ZnONPs. The rats were exposed to diabetes by an infusion of the 170 mg/kg of alloxan monohydrate. These rats were classified in six groups: (I) untreated diabetic control group (Diabetic- Control), (II) treated by 10U/kg of insulin (D-Insulin), (III) treated by 7mg/dl of pure ZnO (D-ZnONPs), (IV) treated by 170 mg/dl of the *E. billardierei* leaf extract (D-Ext), (V) treated by 7 mg/dl of the green fabricated ZnO (D-ZnONPs-Ext), and (VI) normal healthy group (Control). Finally, the effective agents in controlling diabetes (e.g. insulin, total triglyceride (TG), fasting blood glucose (FBS), high-density lipoprotein (HDL), and total cholesterol (TC)) were evaluated. The experimental results showed that the synthesized nanoparticles by using leaf extract, ZnO (E), had excellent potential as an anti-diabetic agent.

Materials and Methods

Zinc (II) nitrate, ethyl alcohol, and sodium hydroxide having analytical grade were used (Merck, Darmstadt, Germany). Alloxan monohydrate ($C_4H_2N_2O_4.H_2O$) was provided from Sigma Aldrich. Insulin level was measured by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Mercodia, Sweden). The kits for exploring the TC, HDL, FBS, and TG were provided from Pars Azmoun Company (PAC, Tehran, Iran). Deionized water (DW) was used throughout fabrication of the ZnO NPs.

Animals

In this research, all the steps were performed according to National Institutes of Health guidelines for the use of laboratory animals. The 30 Wistar rats (5 male rats in 6 groups), about one month old and of 200±70g weight were kept on a standard rodent diet (vitamins 3%, carbohydrates 30%, lipids 12%, proteins 22% and free access to water) under standard laboratory conditions (55±4% humidity, 22 ± 1°C, 12 h light cycles, and 12h dark cycles).

Preparation of the extract

The green leaf of *E. billardieri* was collected from a mountainous region (Ardabil, Iran) and confirmed by a botanist in the University of Mohaghegh Ardabili. Afterward, the plant leaf was washed by DW and dried at 25°C for 5 days. Then, the dried powder of the leaf (10 g) was well-mixed in 100 mL DW, incubated at 95°C for 2 h, and centrifuged at 2500 g for 5 min.

Preparation of the nanoparticles

The pure ZnONPs were fabricated in DW and the ZnO (E) NPs were biosynthesized by the *E. billardieri* leaf extract. For preparation of the ZnONPs, zinc nitrate (3.65g) was dissolved in 100 mL DW at 37°C under stirring. Afterward, NaOH (5 M) was drop-wisely added to the solution until pH adjusted to 10. Then, the suspension was placed in a microwave oven for 10 min. The white precipitate was centrifuged (2000g/6 min), washed by DW and ethyl alcohol, respectively, and dried at 60°C overnight. For the ZnO (E) NPs biosynthesis, the procedure was the same as Experiment 1; the one exception was that zinc nitrate (3.65g) was dissolved in a solution with the following composition: 80 mL DW and 20 mL the *E. billardieri* leaf extract.

Instruments

The detailed characterization instruments are in the supporting materials.

Anti-diabetic activities

After the end of sixteen day of treatment, the rats were sacrificed by Ketamine/Xylazine (80:15 mg/Kg) anesthesia. The rats blood samples were prepared in sterile tubes, held at 4°C for 25 min, and centrifuged at 1700 g for 16 min. The prepared serum was used to estimate the levels of TC, TG, FBS, and HDL. The insulin level was measured by using a highly specific ELISA kit (Mercodia AB, Sweden), using Microplate reader (URIT Medical Electronic Co., Ltd., Guangxi, P.R China).

Results and Discussion

Characterizing of the nanoparticles

The XRD patterns were applied to discover the crystalline structure of the prepared nanoparticles **(Figure 1a).** The ZnONPs synthesized in water showed diffraction peaks that attributed to hexagonal structure (JCPDS card No.: 36–1451) [30,31]. In the case of the ZnO (E) NPs, all the peaks displayed the same XRD pattern (without any shifts in the position of the peaks), but showing the remarkable difference in the peak intensity. Moreover, no diffraction peaks corresponding to the impurities were observed in the XRD patterns, demonstrating that the nanoparticles have high purity. The crystallite size of the ZnO and ZnO (E) NPs were calculated by Scherrer's equation as 34 and 27 nm, respectively. It is clear that the different functional groups presented in the *E. billardieri* extract, are responsible for reduction and stabilization the ZnO (E) NPs in comparison of the ZnO sample [32]. By the EDX analysis, elemental compositions of the samples were identified and displayed in **Figure 1b**. The spectra for the ZnO powder show the presence of O and Zn elements, whereas O, C, and Zn elements were clearly observed in the spectrum of ZnO(E) NPs. According to the presence of carbon element on the ZnO(E) NPs, existence of a binding from the green synthesized ZnO and different functional groups from the extract are indicated. Besides, in accordance with the XRD analysis, the EDX spectra with no other impurities indicated that the samples have been successfully prepared through the proposed simple route.

To observe shape of the prepared nanomaterials, the FE-SEM and TEM images were displayed, as illustrated in **Figure 2**. In the FE-SEM images **(Figure 2a and Figure 2b),** the particles in both samples are observed approximately spherical in shape. The TEM images showed that in the ZnO (E) sample, the bio-compounds of the extract have loaded onto surfaces of the ZnO (E) NPs. In **Figure 2**, the TEM and FE-SEM images shows that similar to the XRD analysis, the ZnO sample formed bigger particles relative to the ZnO (E) NPs. This reduction size of particles in the case of ZnO (E) can be related to the biocompounds presented in extract [33].

The results of the UV–visible spectroscopy of the synthesized

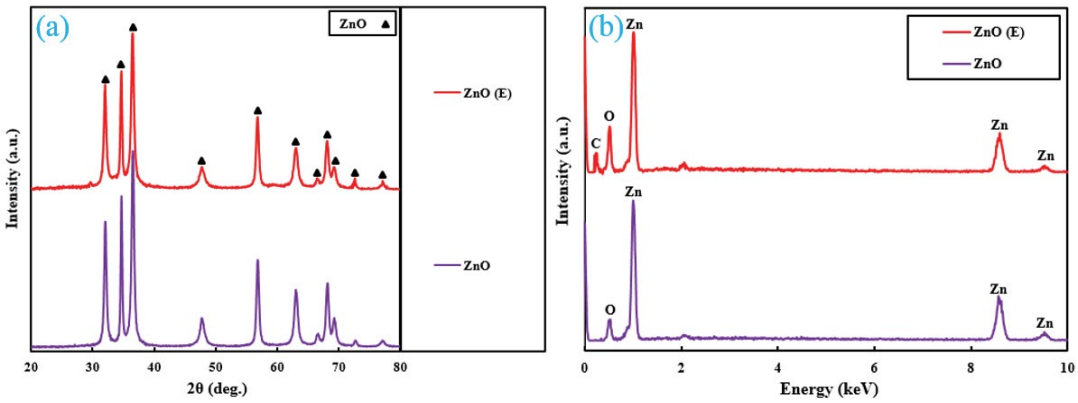

Figure 1 **(a)** XRD patterns, and **(b)** EDX spectra for the ZnO and ZnO (E) NPs.

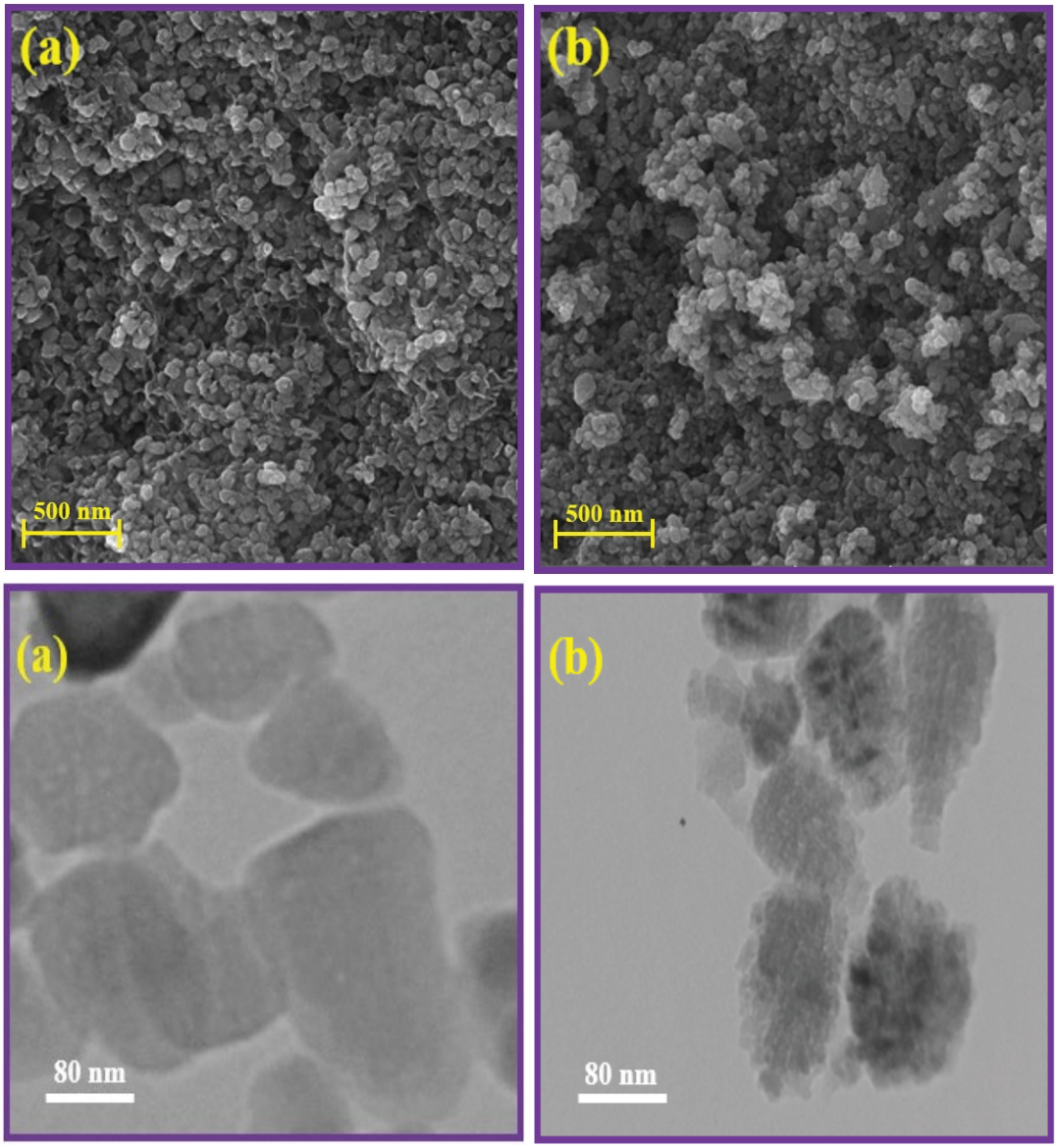

Figure 2 FE-SEM and TEM images for the prepared samples: (a) ZnO and (b) ZnO (E).

nanoparticles are displayed in **Figure 3a**. As confirmed by the spectrum for ZnONPs, one intense absorption in 375 nm was observed [34]. Furthermore, in the ZnO(E) NPs according to the quantum confinement effect, a distinct blue shift relative to the prepared ZnO in water was displayed [20]. In addition, for these nanoparticles in the visible region, a strong absorption was observed due to some bio-molecules of the leaf extract, which is in agreement with the results obtained from the TEM and EDX analyses.

TG analysis curves for the ZnO, ZnO (E) NPs were presented in **Figure 3b**. In the pure ZnO sample, thermal stability was confirmed up to temperature 700 ºC. This sample displayed a weight loss of about 2.5%, which is attributed to desorption of water molecules. In the ZnO (E) NPs, the weight loss up to 230 ºC is ascribed to the dehydration phase. Moreover, increasing the heat of the ZnO (E) NPs caused more weight loss [35]. This decrease can be attributed the join up bio-molecules of the leaf extract on the ZnONPs. The weight percentages of bio-molecules after heating up to 640 ºC were calculated as 16.3%. According to the results of the TG analyses, the results obtained from the DRS and EDX analyses were confirmed.

FTIR analysis in the range of 400 to 4000 cm^{-1} was performed to identify the possible biocompounds in the extract which were responsible for the reduction of ZnONPs formation. FTIR spectra of the ZnO and ZnO (E) NPs as well as *E. billardieri* leaf extract are illustrated in **Figure 3c**. The existence of some peaks at the spectrum of the extract like those occurred in 3422, 1630, and 674 cm^{-1} were attributed to O–H stretching of phenols and alcohols, C═O groups of phenolic acids, flavonoids, and C–H stretching of organic compounds [36]. On the other hand, the peaks at 470, 1024, 1098, 1386, 1604, 2868, 2932, and 3430 cm^{-1} in ZnO and ZnO (E) NPs are characteristics of Zn–O bond, C–OH stretching vibrations, C–O bonding, C═C of aromatic rings, C═O groups of flavonoids, C–H stretch of alcohols, C–H stretch of phenols, and O–H stretching vibration, respectively [30,35].

Anti-diabetic activities

Anti-diabetic activity of the prepared samples was investigated on the diabetic rats. Accordingly, blood sampling was performed after the end of sixteen day of treatment and FBS, HDL, TG, TC, and insulin were measured. The differences in some results between the fabricated nanoparticles were statistically significant by one–way ANOVA at P <0.05. **Figure 4a** displayed anti-diabetic

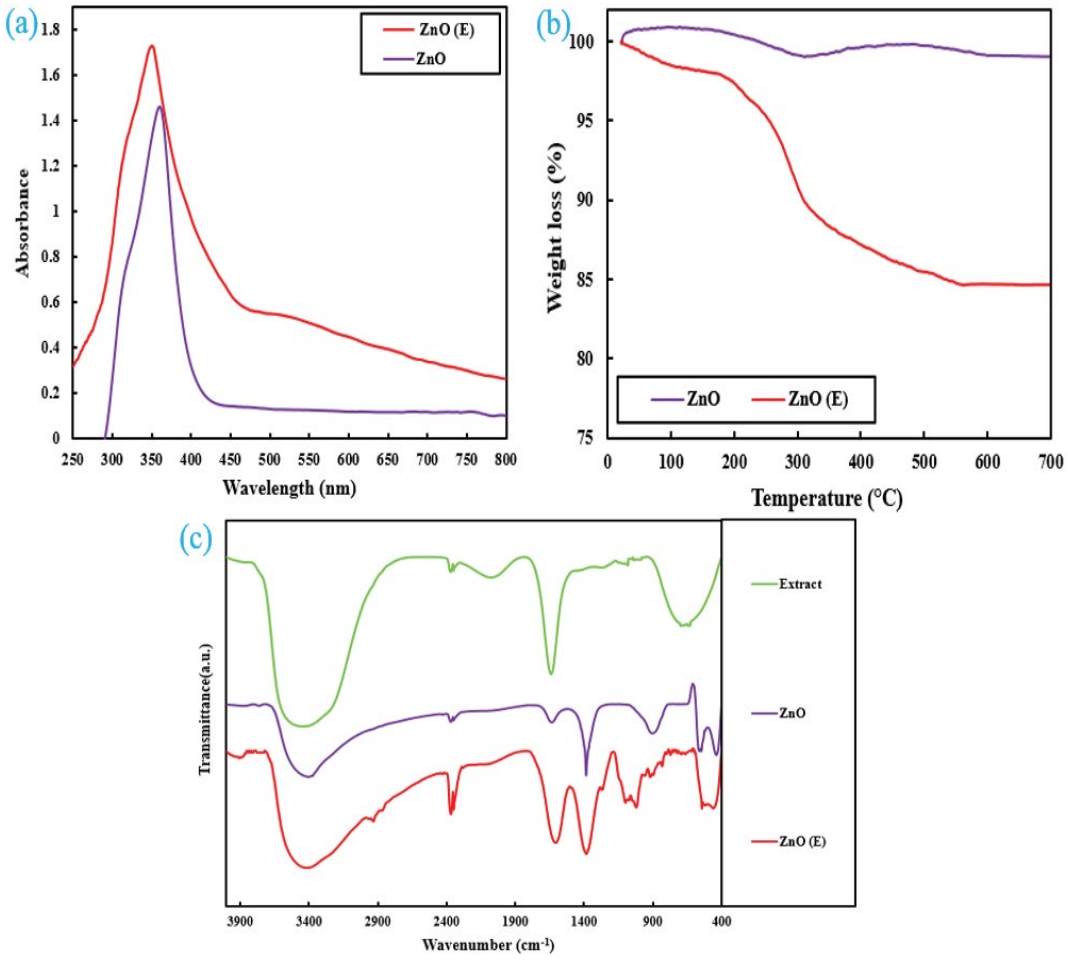

Figure 3 **(a)** UV–VIS DRS spectra, **(b)** TG and **(c)** FT-IR spectra analyses for the fabricated samples.

treatments on FBS levels. As observed, in all the treated groups, an abundant drop in the FBS level was detected. Furthermore, the highest reduction in the FBS level with the D-ZnONPs-Ext followed by D-ZnONPs, D-Ext and D-Insulin treatments was obtained. The decline in the FBS can be ascribed to the effect of ZnONPs on the glucose metabolism and the amount of insulin increment [37,38]. Moreover, the pure ZnO NPs showed a weak anti-diabetic activity as compared to green synthesized ZnO NPs that is due to the smaller size, better penetration, and larger surface area of the ZnO (E) NPs [35]. In addition, the anti-diabetic activity of the *E. billardieri* extract can be attributed to the presence of several biocompounds such as flavonoids and alkaloids [26,36]. The similar results were obtained in treated diabetic rats by green fabricated ZnO NPs [14]. The resulting extract and fabricated nanoparticles on the studied groups for assessment the changes insulin level were reused **(Figure 4b).** According to **Figure 4b**, the insulin level in the D-Control group shows the significant decrease as compared with the control group. Furthermore, in the groups treated with D-ZnONPs-Ext and D-Insulin samples as compared with the D-Control group, the insulin levels were significantly

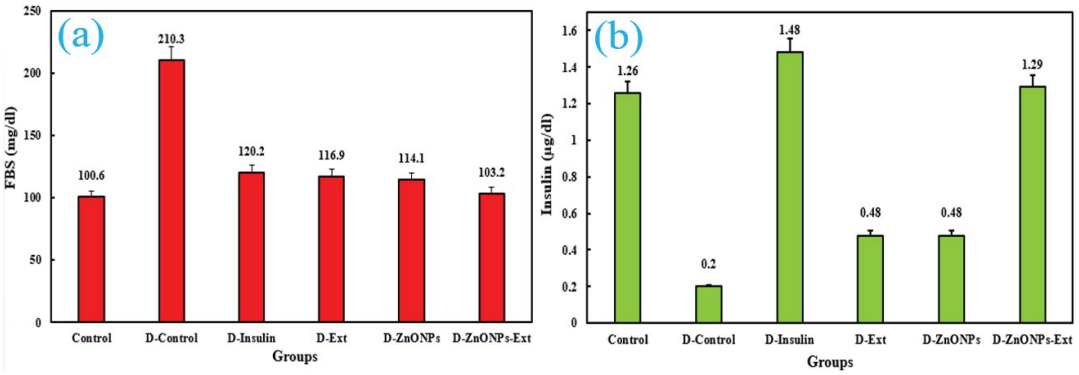

Figure 4 **(a)** Changes in blood glucose, and **(b)** changes insulin level in the studied groups.

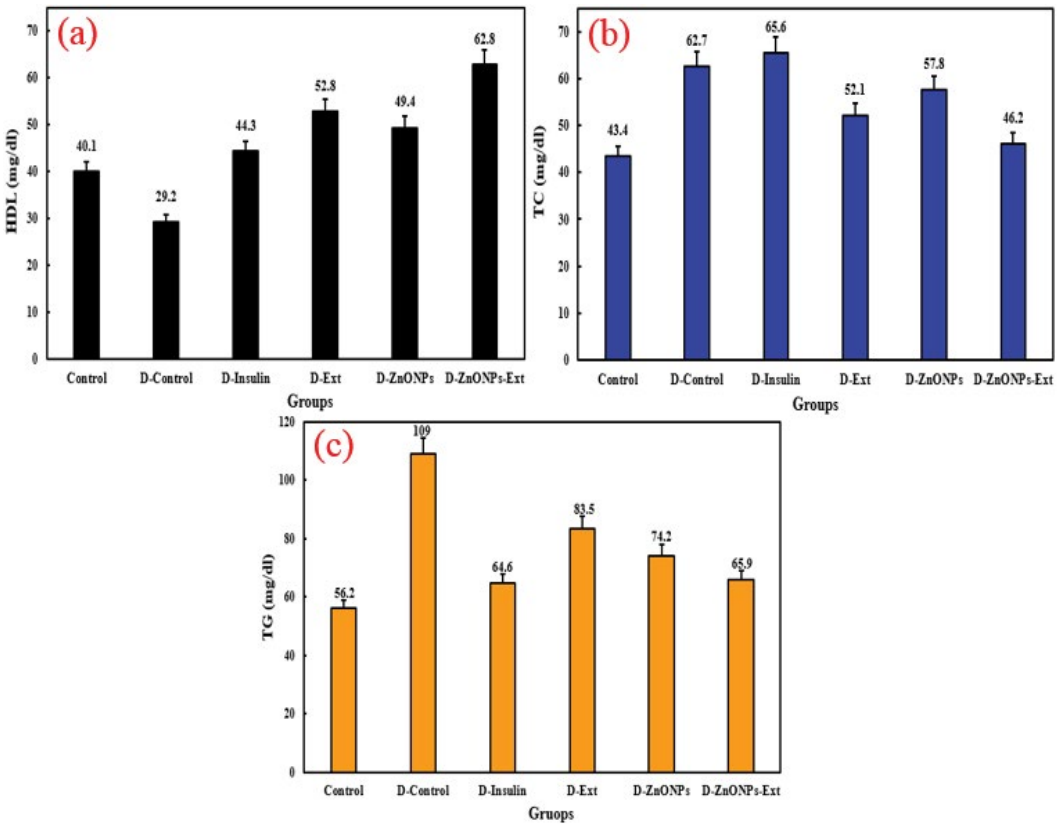

Figure 5 **(a)** Changes in HDL, **(b)** Changes in TC, and **(c)** Changes in TG level in the studied groups.

improved. Moreover, no significant difference was discovered between the groups treated with ZnONPs-Ext and D-Insulin. On the other hand, the zinc shortcoming in the pancreas has positively correlated by type 2 diabetes. This deficiency can adversely affect in the secretion ability of the pancreatic β-cells and insulin production [38]. Therefore, the anti-diabetic action in the ZnONPs-Ext sample could be attributed by increased insulin secretion amount from the β-cells [39,40].

Hyperlipidemia is an important parameter in the diabetes [41], thus in the serums of the studied groups, lipid profile levels such as HDL, TG, and TC were assessed and displayed in **Figure 5**. According to the **Figure 5a**, the diabetic rats before receiving any treatment shows low HDL level, while the rats that received ZnO NPs-Ext shows an increase in the HDL level. Furthermore, in the diabetic rats, the TG and TC levels were increased before receiving any treatment **(Figure 5b and Figure 5c).** The groups that received ZnO NPs-Ext displayed a significant reduction in TG. The samples effect on the serum TC levels of the studied groups was also not significant as compared with the D-Control rats. In this research, an increased blood sugar level followed by increased TC and TG levels causes a decreased HDL. A low level of insulin followed by a high level of blood glucose can indirectly build up cholesterol, and triglyceride; as a result, it reduces the HDL levels [42]. The ZnO can act like α-blocker; hence it has an important role in enzymatic activities [43]. The results showed that the lipid profile levels were improved after treatment by ZnO NPs.

Conclusion

Due to the problems arising from diabetes as a metabolic disorder, it is associated with several complications which require great efforts to be treated. In this research, ZnO nanomaterials were fabricated by green synthesis procedure and their anti-diabetic activities were assessed. The EDX, UV-vis DRS, FT-IR and TG analyses showed existence of the bio-molecules from *E. billardieri* leaf extract on the ZnO (E) NPs. Furthermore, the ZnO (E) NPs in comparison to the pure ZnO displayed an increased treatment effect on the tested diabetic male rats. Moreover, the ZnO (E) sample were excellent effective in the lessen FBS level and have excellent control the levels of some lipids. Based on the results obtained, the prepared of the ZnO (E) sample were help for effective upgrade of the HDL levels. As compared with ZnONPs prepared in water, the ZnO (E) NPs fabricated by *E. billardieri* leaf extract were much influential in reducing of the TG and TC levels. Due to the promising attributes of the fabricated green ZnO (E) NPs and their favorable anti-diabetic efficacy in the treatment of diabetic rats, these green nanoparticles can be introduced as nano-anti-diabetic drugs.

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