



Assessment of the anti-diabetic effect of *Bauhinia variegata* gold nano-extract against streptozotocin induced diabetes mellitus in rats

Abeer Hamed Abdel-Halim^{1*}, Amal Abdel Azeem Fyiad¹, Wael Mahmoud Aboulthana¹, Nermin Mohammed El-Sammad¹, Ahmed Mahmoud Youssef², Mamdouh Moawad Ali¹

¹Biochemistry Department, Genetic Engineering and Biotechnology Division, National Research Centre, 33 Bohouth St., P.O. 12622, Dokki, Giza, Egypt.

²Packaging Materials Department, Chemical Industries Research Division, National Research Centre, Dokki, Giza, Egypt.

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ABSTRACT

Diabetes mellitus (DM) is the most common type of the metabolic endocrine disorders. It is categorized as the third cause of death after heart and cancer diseases. Due to the undesirable side effects of the synthetic anti-diabetic drugs, the medicinal plants were selected for DM treatment. The recent studies directed to use nanotechnology in medicine to overcome restrictions of the drugs that were represented by weak bioavailability, insolubility in water, low absorption by the intestine, and inability to reach to the appropriate site of action. The study aimed to investigate the antioxidant, antidiabetic, and hypolipidemic effect of *Bauhinia variegata* (*B. variegata*) extract before and after incorporating gold nanoparticles (Au-NPs) against DM induced by streptozotocin (STZ) in rats. The present study conducted on 36 male albino Western rats divided into six groups {control, *B. variegata* extract treated group, *B. variegata* gold nano-extract treated group, diabetic group [injected by STZ intrapretinually (i.p.) at a dose of 60 mg/kg], diabetic rats treated with *B. variegata* extract, and *B. variegata* gold nano-extract groups}. The DM related biochemical functions (liver and kidney functions, glucose, insulin, and lipid profile) were assayed. Moreover, the enzymatic and non-enzymatic antioxidants were assayed in addition to lipid peroxidation products in liver and pancreas. Furthermore, the native protein, lipoprotein patterns, and isoenzymes were electrophoretically studied. It was found that the administration of both *B. variegata* extract and nano-extract to diabetic rats ameliorated the deleterious effects that occurred as a result of STZ injection and restored the biochemical functions in addition to levels of hepatic and pancreatic antioxidants to normalcy. These findings were supported by the histopathological examination. It was concluded that *B. variegata* nano-extract exhibited more antidiabetic effect through restoring the normal architecture of pancreatic β -cells in addition to the antioxidant and hypolipidemic effect than extract alone, which indicated that the efficacy of *B. variegata* extract was increased after the incorporation of Au-NPs.

INTRODUCTION

Diabetes Mellitus (DM) belongs to the metabolic disorders that are characterized by disrupting metabolism of proteins, carbohydrates, and fats with an increase in blood glucose levels. Subsequently, it leads to damage of different organs, including heart, kidneys, and eyes (Surya *et al.*, 2014; Kulkarni and Garud, 2016; Aboulthana *et al.*, 2018).

Two types of DM were well known. Type I is insulin dependent and occurred as a result of either partial or complete insufficient insulin secretion. Type II diabetes is called insulin resistance and characterized by low sensitivity of tissue to insulin. About 90% of DM patients are suffering from Type II diabetes and therefore, it is considered as the commonest form of diabetes (Kumar *et al.*, 2015; Kulkarni and Garud, 2015). It represents the third reason for death, following the heart and cancer diseases (Guariguata, 2011). The number of diabetic patients was about 382 million in 2013 and predicted to increase to 592 million by 2035 (Sheweita *et al.*, 2016).

Streptozotocin (STZ) is a naturally occurring nitrosourea product synthesized by a strain of the soil microbe *Streptomyces*

*Corresponding Author
Abeer Hamed Abdel-Halim, Department of Biochemistry, National Research Centre, Dokki 12622, Egypt. E-mail: abeer.hamed@yahoo.com

achromogenes (Vivek, 2010). It is an unusual aminoglycoside containing a nitrosoamino group that enables the metabolite to act as a nitric oxide donor. It is widely used to induce permanent diabetes in rats due to its ability to destroy β cells and insulin-secreting pancreatic endocrine cells directly (Kumar *et al.*, 2015). Current use of STZ is mostly as an investigational drug for diabetes research (Ali and Agha, 2009). It induces hyperglycemia during 72 hours associated with various side effects as injury of liver and kidney tissues associated with bone marrow depression (Brenna *et al.*, 2003; Akbarzadeh, 2007).

Several studies suggested recently that free radicals play a key role in the pathogenesis of diabetes. Therefore, drugs that have antioxidant capacity could be effective in reducing diabetic complications as antipathies, cardiovascular disorders, blindness, renal failure, neuropathies, and cancers (Roy *et al.*, 2011; Sheweita *et al.*, 2016). The available synthetic drugs for DM are cost effective and associated with different side effects, hence searching for alternative drugs that can overcome these problems, is considered as a persistent need (Balde *et al.*, 2015). Plants were and still an important source for antidiabetic drugs where they deal with our bodies without side effects (Gandhi and Sasikumar, 2012). *Bauhinia variegata* L. (*B. variegata*) (also known as orchid tree, camel's foot tree, mountain ebony, or Kanchnar) is one of these plants that used in traditional medicine, especially in India. It belongs to the family Fabaceae. They have different biological activities, including anti-inflammatory, antimicrobial, anti-diabetic, antitumor, analgesic, astringent, and diuretic effects (Rashid, 2014; Kulkarni and Garud, 2015). Different parts of *B. variegata* are rich in active phyto-constituents as glycosides, flavonoids, steroids, and terpenes, which exhibit high biological activities.

It was reported that *B. variegata* leaves contain isoquercetin, quercetin 3-methyl ether, naringenin, rutin, and luteolin (Kulkarni and Garud, 2016). In addition, ethanolic *B. variegata* leaves extract is rich in insulin like protein, which has a partial identical amino acid sequence to the bovine insulin that exhibit an effective role in lowering level of blood glucose (Rashid, 2014).

Most of these biologically active components absorbed slowly due to their high molecular weights that decrease their ability to cross the cellular lipid membrane and subsequently lead to decrease in their efficacy and bioavailability. Therefore, the integration of the traditional medicinal plants with nanotechnology can overcome such problems where it can increase their bioavailability, avoid repeated administration, and reduce their toxicity. So that, the drugs at the nano-scale can achieve this purpose for increasing the activity and therapeutic effect of extracts (Prasad, 2014; Bonifácio *et al.*, 2014; Mamillapalli *et al.*, 2016). From this point of view, this study was designed to select the most effective plant extract to be incorporated by gold nanoparticles (Au-NPs), and hence to investigate its antioxidant, anti-diabetic, and hypolipidemic effect against STZ-induced DM in rats.

MATERIALS AND METHODS

Chemicals

STZ, Commassie Brilliant Blue G-250, Sudan Black B (SBB), Fast Blue RR, and all other chemicals were procured from Sigma-Aldrich (St. Louis, MO).

Preparation of plant extract

Young *B. variegata* L. (var. Candida) leaves were freshly collected from Orman garden then dried in an incubator at 50°C for 72 hours. The dried leaves were consequently crushed into powdered materials using an electric blender then percolated with different solvents (methanol, ethanol, water, and ethyl acetate) according to the protocol documented by Gaber and Elsayed (2010) at room temperature for 3 days. The residues were obtained through evaporating the different extracts in oven at 37–40°C.

Determination of total polyphenols and antioxidant activity

The biological efficiency was evaluated through quantifying the total polyphenolic compounds (Singleton and Rossi, 1965), total antioxidant capacity (Prieto *et al.*, 1999), and total reducing power (Oyaizu, 1986) in addition to percentage of the antioxidant activity that was assayed using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) as free radicals initiator (Brand-Williams *et al.*, 1995) in the different plant extracts to select the most suitable and effective one.

Synthesis of gold nanoparticles (Au-NPs)

The Au-NPs was commonly fabricated by the chemical reduction method through two main parts. The first part was carried out by reduction of Au^{+3} (HAuCl_4) to Au^0 through the reaction between tetrachloroauric acid (HAuCl_4) and trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$) in an aqueous solution. The second part was carried out to avoid the particles aggregation by stimulating the stabilization using cetyltrimethylammonium bromide (CTAB) (Zhao *et al.*, 2013). Finally, the Au-NPs with narrow size distribution were synthesized and Zeta sizer, transmission electron microscopy (TEM) and x-ray diffraction pattern (XRD) were used to characterize and determine the size and size distribution of the synthesized gold nanoparticles.

Preparation of cellulose nanocrystal (CNC)

Alkali solution (4% W/V NaOH) was added to round bottom flask containing a desired amount of dried rice straw powder under reflux condition at 100–120°C for 2 hours. After the filtration of the mixture, it was washed several times with distilled water to remove hemicellulose and lignin dissolved in the solution. The resulting fiber was dried to be used for the bleaching treatment that was performed by adding desired weight (30 g) of the dried fiber into 200 ml of solution consisting of NaClO_2 (1.7%), acetic buffer and distilled water under reflux condition at 110–130°C for 4 hours. The mixture was filtered after cooling then washed with distilled water for obtaining the white cellulose that was consequently dried at –39°C for 24 hours using freeze dryer (Labconco). CNC was prepared by adding approximately 5% of cellulose fiber to sulfuric acid solution (65-wt.% H_2SO_4) under fixed time and temperature conditions at 45°C for 45 minutes for achieving optimum yield. After sulfuric acid hydrolysis, the hydrolyzed cellulose was washed five times by centrifuging the solution at 10,000 rpm for 10 minutes for removing the excess sulfuric acid. The resulting suspension of CNC was dialyzed against distilled water until achieving constant pH. The resultant CNC suspension was stored in refrigerator until to be used.

Preparation of *B. variegata* gold nano-extract

Bauhinia variegata nano-emulsion was prepared using *B. variegata* crude ethanol extract, non-ionic surfactant Tween20 (HLB-16.7), CNC, and water via spontaneous emulsification method. Nano-emulsion was carried out in two steps: in the first step, organic phase was fabricated through mixing *B. variegata* crude sample with chosen surfactant (Tween20) in the following ratio (1:5). This was followed by adding 3 g of CNC to the mixture that was consequently sonicated for 30 minutes. In the second stage, the organic phase (*B. variegata*, Tween 20 and CNC) was added drop-by-drop (20ml/min) to water using separating fennel and stirring the system magnetically (800 rpm) at 60°C for 5 hours. Then, the prepared gold nanoparticles (Au-NPs) were added to the prepared nano-emulsion by the following ratio (1%). The mixture was sonicated to another 30 minutes at 50°C.

Characterization of the prepared Au-NPs

XRD

Crystal structure of the synthesized Au-NPs was determined by a Philips X-ray diffractometer (PW 1930 generator, PW 1820 goniometer) that equipped with Cu K α radiation (45 kV, 40 mA, with $\lambda = 0.15418$ nm). The analysis scans were run in 2 θ range of 5–80° with 0.02 step size and 1 second step time.

TEM

The synthesized Au-NPs were characterized morphologically in addition to determining the particles size by using TEM (JEM-1230, Japan) operated at 120 kV with resolution until 0.2 nm and maximum magnification of 600 \times 10³. Before characterization, aqueous sample dispersion (one drop) was placed on a carbon-coated copper grid then allowed in air to dry.

Median lethal dose of *B. variegata* extract and nano-extract (LD₅₀)

The ethanolic *B. variegata* extracts (before and after incorporating Au-NPs) were evaluated for calculating the LD₅₀. One hundred and twelve adult albino Western rats (weighting 150–180 g) were used. They divided into two sets of seven groups (eight rats/group) for evaluating the LD₅₀ of both *B. variegata* extract and nano-extract. Mortality was recorded after 24 hours of oral administration of extract and nano-extract with rising doses (10, 20, 30, 40, 50, 60, and 70 ml/kg). The LD₅₀ was calculated according to the equation suggested by Paget and Barnes (1964). It was found that the LD₅₀ values of the *B. variegata* extract and gold nano-extract were found to be 36.50 and 51.5 ml/kg. Therefore, the doses used for the therapeutic investigation were determined as 1/20 LD₅₀ for the extract and nano-extract (1.83 and 2.58 ml/kg, respectively).

Animals

Thirty-six healthy male albino Western rats (weighting 150–180 g) were housed for 7 days in our laboratory in National Research Centre, Dokki, Giza, Egypt, where they maintained under normal nutritional and environmental conditions (12 hours light/dark cycles and 25 \pm 2°C). The experimental protocols for the studying and handling of animals were performed according to

the Institutional Animal Ethical Committee of National Research Centre (No: 19070) and was conducted in accordance with “Guide for the care and use of laboratory animal.”

Induction of diabetes mellitus

Rats were fasted overnight and injected intraperitoneally (i.p.) by single dose of freshly prepared STZ solution in citrate buffer (100 mM/l at pH 4.5) at a dose of 60 mg/kg b.w. (Archana *et al.*, 2001). After 72 hours of STZ administration, the blood was collected from tail vein for estimating fasting blood glucose level using Accu-Chek sensor (comfort glucometer (China). Rats were considered as diabetic when blood glucose level exceeded 250 mg/dl.

Experimental design

Animals were housed in six cages (six rats per cage) and randomly divided as following:

- Control group: Rats were fed with normal diet and received tap water.
- *Bauhinia variegata* extract treated (E) group: Rats were orally treated with ethanolic *B. variegata* extract at a dose of 1.83 ml/kg (1/20 LD₅₀).
- *Bauhinia variegata* gold nano-extract treated (N) group: Rats were orally treated with *B. variegata* gold nano-extract at a dose of 2.58 ml/kg (1/20 LD₅₀).
- Diabetic (D) group: Rats were i.p. injected with a single dose of STZ and then diabetic rats were sacrificed after 1 week of STZ injection.
- Diabetic group treated with *B. variegata* extract (D+E): Rats i.p. injected with STZ then treated with ethanolic *B. variegata* extract at a dose of 1.83 ml/kg.
- Diabetic group treated with *B. variegata* gold nano-extract (D+N): Rats i.p. injected with STZ then treated with *B. variegata* gold nano-extract at a dose of 2.58 ml/kg.

Collection of samples

At the end of the experiment (4 weeks), rats were fasted for 18 hours and anesthetized by slight exposure to diethyl ether then sacrificed by cervical dislocation. Blood samples were drawn from retro-orbital plexus and divided into two parts. Part I was deposited in NaF glucose vacuum tubes (Becton Dickinson, New York, NJ) for glucose estimation. Part II was allowed to clot at room temperature, centrifuged for 15 minutes at 3,000 rpm, and then serum was separated and kept in at –20°C until used for the biochemical assays. After sacrificing the animals by cervical dislocation, the pancreas and liver tissues were excised and divided into three portions. A small portion was immediately preserved in 10% neutral buffered formalin solution for histopathological investigation. The second portion was homogenized in potassium phosphate buffer (pH 7.4) using Tissue Master TM125 (Omni International, USA). The liver and pancreas tissue homogenates were centrifuged at 3,000 rpm for 10 minutes, and the clear supernatant was stored at –80°C to be used for biochemical assays. The last portion of liver and pancreas tissues was rapidly frozen in liquid nitrogen for electrophoretic analysis.

Biochemical analysis

Glucose level, liver enzymes [serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP)], concentrations of Total Protein (TP), Kidney functions (Urea, Creatinine), as well as lipid profile [Total Cholesterol (TC), Triglycerides (TGs), and HDL-cholesterol] were assayed using commercially available kits (Spectrum Diagnostics Egyptian Company for Biotechnology, Cairo, Egypt). Moreover, LDL-cholesterol was calculated by the formula of Schumann and Klauke (2003)

$$\text{LDL} = \left[(\text{TC} - \text{HDL}) - \frac{\text{TG}}{5} \right]$$

In addition, insulin level was quantified in plasma using sandwich enzyme-linked immuno sorbent assay (ELISA) following instruction of the kit (ELISA, Boehringer Mannheim, Mannheim, Germany).

Reduced glutathione was estimated in hepatic and pancreatic tissues according to the method described by Beutler *et al.* (1963). Catalase and Superoxide Dismutase activities were evaluated in liver and pancreas according to the methods of Aebi (1984) and Minami and Yoshikawa (1979), respectively. Thiobarbituric acid reactive substances (TBARS) were estimated as a marker for lipid peroxidation in hepatic and pancreatic tissues according to method suggested by Lefevre *et al.* (1998).

Statistical analysis

The statistical analysis was carried out by one-way analysis of variance test followed by Bonferroni test using Statistical Package for Social Sciences (SPSS for windows, version 19.0). The results were expressed in tables and figures as mean \pm standard error (SE). The differences were considered as statistically significant when a “*p*” value was less than 0.05.

Native electrophoretic patterns

Protein patterns

The pancreas and liver tissues were rapidly frozen in liquid nitrogen and homogenized in Tris-HCl buffer (0.01 M and pH 7.4). The clear supernatants were transferred to new tubes after centrifuging the homogenates at 10,000 rpm for 15 minutes. Equal volumes of the individual supernatants in each group were mixed together in one tube and used as one sample. The concentration of TP was assayed in all samples (Bradford, 1976). During the electrophoretic assays, the samples loaded in all wells with equal protein concentrations.

Native proteins were electrophoretically separated using Polyacrylamide Gel Electrophoresis based on the methods

documented by Hames (1990) and modified by Darwesh *et al.* (2015) who reported that samples, gels, and running buffers contained no sodium dodecyl sulfate. The native protein bands were stained by Commassie Brilliant Blue G-250 and visualized as blue bands. The lipid moieties of native protein were stained by SBB visualized as black bands (Subramaniam and Chaubal, 1990).

Isoenzymes

The isoenzymes were electrophoretically detected through the identification of the enzyme subunits. First, electrophoretic catalase pattern (CAT) and electrophoretic peroxidase (POX) pattern were assayed for native gel after electrophoretic run by incubating with H₂O₂ as the substrate then stained. The stained CAT subunits appeared as yellow bands in the gel (Siciliano and Shaw, 1976). While, the stained POX subunits appeared as brown bands in the gel (Rescigno *et al.*, 1997). Moreover, α -amylase was electrophoretically detected according to the method suggested by Rammesmayer and Praznik (1992) through incubation of the gel with a solution containing soluble starch followed by staining with iodine solution.

The polyacrylamide gel plates were scanned then analyzed by the Quantity One software (Version 4.6.2) that was used to determine the relative mobility (Rf), band percent (B%) and band quantity (Qty) of the bands separated electrophoretically. In addition, the similarity index (SI%) and genetic distance (GD%) were calculated to compare all treated groups to control group.

Histopathological examination

After sacrifice, small specimens from pancreatic and hepatic tissues were autopsied and immediately preserved in 10% neutral buffered formalin solution. The tissue specimens were washed by tap water after 24 hours and dehydrated in serial dilutions of alcohol solutions, cleared in xylene, and embedded in paraffin at 56° in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides and deparaffinized then stained by hematoxylin and eosin (H&E) stain to be examined histopathologically under light microscope according to the method suggested by Bancroft *et al.* (1996).

RESULTS

Selection of the most effective *B. variegata* extract and integration of Au-NPs

As revealed in Table 1, it was found that the ethanolic *B. variegata* extract was the most effective extract noticed with the highest concentration of total polyphenols (741.28 \pm 4.82 mg

Table 1. Polyphenol concentration, total antioxidant capacity and free radical scavenging activity in different *B. variegata* extracts.

| Solvent | Polyphenol (mg gallic acid/100gm) | Total antioxidant capacity (mg gallic/gm) | Reducing power (μ g/mL) | Antioxidant activity (%) |
|-----------------|--------------------------------------|--|------------------------------------|-----------------------------|
| Methanol | 729.02 \pm 3.45 | 3.98 \pm 0.036 | 11.93 \pm 1.51 | 72.4 % |
| Ethanol* | 741.28 \pm 4.82 | 5.32 \pm 0.13 | 12.94 \pm 1.63 | 78 % |
| Ethyl acetate | 722.76 \pm 4.11 | 1.69 \pm 0.08 | 9.31 \pm 1.20 | 66 % |
| Water | 619.90 \pm 9.50 | 0.77 \pm 0.03 | 9.15 \pm 1.15 | 76 % |

Values expressed as mean \pm SE of four replicates, *: The most effective extract with respect to the others.

gallic acid/100 g), total antioxidant capacity (5.32 ± 0.13 mg gallic acid/g), iron reducing power (12.94 ± 1.63 $\mu\text{g/mL}$), and free radical scavenging activity (78%).

Based on the *in vitro* experiments that used to measure the antioxidative efficiency of active ingredients, the ethanolic *B. variegata* extract was selected to be incorporated by Au-NPs according to the method suggested by Elia *et al.* (2014). It was found that the incorporation of Au-NPs enhanced efficiency of the ethanolic *B. variegata* extract through increasing concentration of total polyphenols, total antioxidant capacity, iron reducing power, and free radical scavenging activity (827.76 ± 8.94 mg gallic acid/100 g, 8.87 ± 0.79 mg gallic acid/g, 19.22 ± 1.94 $\mu\text{g/mL}$, and 82%, respectively) as depicted in Table 2.

Studying the structural properties of prepared Au-NPs

The XRD and TEM are the most suitable techniques used for studying structural properties of the materials synthesized at nano-scale. The prepared Au-NPs were examined through the XRD diffraction pattern. The XRD data revealed that the fabricated Au-NPs achieved in presence of AuCl_4^- analogous diffraction peaks are allocated to metallic Au phase with the greatest significant representative peaks which seem at 38° , 43.8° , and 65° attributed to the crystallographic planes (1 1 1), (2 0 0), and (2 2 0), respectively. The peak width of Au-NPs from crystalline plane (1 1 1), sizes of the Au crystallite were found to be approximately 15 nm for Au-NPs (Youssef *et al.*, 2014).

Moreover, the morphology of the prepared Au-NPs was examined by the TEM, which determine the shape and size of gold nanoparticles. The size of Au-NPs has been examined by determining the diameter of whole particles on TEM images. The average width of gold was in the range of 15 nm with very little particles of higher and lower size distribution. In addition, the TEM data displayed that the Au-NPs is that greatest of the gold nanoparticles are round or spherical in shape as shown in Figure 1.

Biochemical studies

Data compiled in Table 3 revealed that the final body weight decreased by 21.8% than initial body weight in diabetic group. While, after treatment the antidiabetic rats with *B. variegata* extract and *B. variegata* gold nano-extract, the body weight was improved where; the final body weight was increased than initial body weight in both groups by 8.88% and 13.43%, respectively.

As shown in Table 4, it was found that there was significantly ($p < 0.000$) elevation in glucose level associated with significant ($p < 0.000$) decline in insulin level in diabetic rats as compared to control group. While, the treatment with ethanolic *B. variegata* extract and gold nano-extract decreased glucose level with significant elevation in insulin level ($p < 0.000$) comparing to diabetic group. In addition, it was found that STZ caused significant ($p < 0.000$) increment in levels of TC, TG, and

LDL-c with significant ($p < 0.000$) decline in HDL-c level when compared with control group. While after the treatment with both extracts, the lipid profile was improved (Table 4).

It was found that levels of AST, ALT, ALP, urea, and creatinine were significantly ($p < 0.000$) elevated and TP level was significantly ($p < 0.000$) decreased in diabetic group (Table 5) with respect to control group. While the treatment with ethanolic *B. variegata* extract and gold nano-extract exhibited hepato- and reno-protective effect through lowering measurements of the liver and kidney functions significantly ($p < 0.000$) as compared to diabetic group.

During the current study, it was observed that levels of the enzymatic and non-enzymatic antioxidants (SOD, CAT, and GSH) in hepatic and pancreatic tissues decreased significantly ($p < 0.000$) associated with significant ($p < 0.000$) elevation of lipid peroxidation product (TBARS) in diabetic group with respect to control group (Table 6). The treatment with either extract or gold nano-extract improved antioxidant enzymes, GSH, and decreased TBARS level in both liver and pancreas as compared to diabetic group.

Native electrophoretic protein and lipoprotein patterns

As illustrated qualitatively in Figure 2a, the native protein pattern was expressed electrophoretically in the healthy

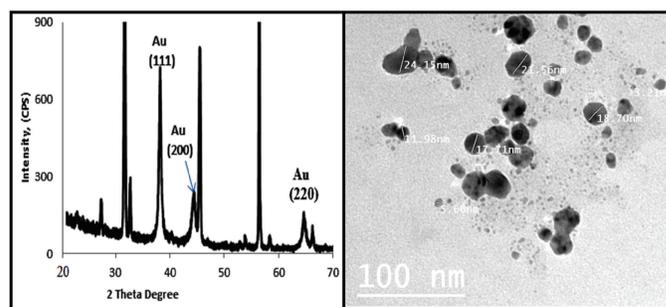


Figure 1. X-ray diffraction spectrums of Au-NPs as well as Transmission Electron Microscope image of Au-NPs.

Table 3. The effect of *B. variegata* extract before and after incorporating gold nanoparticles on initial and final body weights.

| | Initial weight (g) | Final weight (g) |
|-----------------------|--------------------|-------------------|
| Control group | 200 \pm 6.95 | 239 \pm 12.32 |
| Extract-treated group | 186.3 \pm 2.9 | 229.6 \pm 4.7 |
| Nano-treated group | 197.5 \pm 5.44 | 242.65 \pm 9.79 |
| Diabetic group | 188.17 \pm 2.77 | 147.28 \pm 8.46 |
| D+E treated group | 226.7 \pm 6.15 | 246.83 \pm 3.23 |
| D+N treated group | 228.33 \pm 7.5 | 259 \pm 2.66 |

Values were expressed as mean \pm standard error.

Table 2. Polyphenol concentration, total antioxidant capacity and free radical scavenging activity in ethanolic *B. variegata* extract before and after incorporating Au-NPs.

| Ethanol Extract | Polyphenol (mg gallic acid/100gm) | Total antioxidant capacity (mg gallic acid/gm) | Reducing power ($\mu\text{g/mL}$) | Antioxidant activity (%) |
|-----------------|-----------------------------------|--|-------------------------------------|--------------------------|
| Before | 725.44 \pm 5.72 | 6.12 \pm 0.14 | 14.96 \pm 1.23 | 73 % |
| After* | 827.76 \pm 8.94 | 8.87 \pm 0.79 | 19.22 \pm 1.94 | 82 % |

Values expressed as mean \pm SE of four replicates, *: The most effective extract in comparison to the others.

Table 4. The effect of *B. variegata* extract before and after incorporating gold nanoparticles against STZ-induced diabetes on glucose, insulin levels and lipid profile.

| | | Control | Extract treated | Nano-treated | Diabetic | D+E treated | D+N treated |
|----------------------|----------------------|------------|-----------------|--------------|--------------------------|-------------------------|--------------------------|
| Glucose | | 99±4.7 | 105.83±4.42 | 95±5.39 | 318±18.97 ^a | 144.5±4.2 ^{ab} | 127.8±1.82 ^b |
| Insulin | | 0.98±0.06 | 1.02±0.05 | 1±0.04 | 0.53±0.03 ^a | 0.75±0.02 ^{ab} | 0.87±0.02 ^b |
| Lipid profile | TC (mg/dL) | 69.96±0.81 | 71.33±0.75 | 68.74±1 | 107.82±3.7 ^a | 87±0.93 ^{ab} | 80.89±0.74 ^{ab} |
| | TG (mg/dL) | 43.7±0.95 | 43.84±1.12 | 44.89±1.6 | 102.54±2.68 ^a | 61.1±0.65 ^{ab} | 51.73±0.42 ^{ab} |
| | HDL-c (mg/dL) | 30.97±0.8 | 31.86±0.54 | 32.85±0.79 | 19.1±0.51 ^a | 22.45±0.5 ^{ab} | 28.45±0.56 ^b |
| | LDL-c (mg/dL) | 30.25±0.8 | 30.71±1.01 | 28.58±0.86 | 68.22±3.63 ^a | 52.51±1.0 ^{ab} | 41.28±0.6 ^{ab} |

Values were expressed as mean ± standard error, a: significant difference from control group, b: significant difference from toxic group at p<0.000.

Table 5. The effect of *B. variegata* extract before and after incorporating gold nanoparticles against STZ-induced diabetes on liver and kidney functions

| | | Control | Extract treated | Nano-treated | Diabetic | D+E treated | D+N treated |
|-------------------------|---------------------------|-------------|-----------------|--------------|--------------------------|--------------------------|-------------------------|
| Liver functions | AST (U/L) | 34.92±1.22 | 34.88±1.27 | 33.95±0.73 | 95.38±4.2 ^a | 53.02±1.04 ^{ab} | 43.12±1.1 ^{ab} |
| | ALT (U/L) | 30.6±1.22 | 29.1±1.93 | 29.86±1.97 | 79.77±2.24 ^a | 46.39±1.34 ^{ab} | 41.2±1.14 ^{ab} |
| | ALP (U/L) | 102.24±3.85 | 106.2±5.57 | 105.28±2.43 | 228.6±10 ^a | 137.2±2.56 ^{ab} | 131±2.6 ^{ab} |
| | TP (g/dL) | 8.77±0.41 | 9.48±0.43 | 9.14±0.36 | 2.45±0.34 | 4.92±0.26 ^{ab} | 6.74±0.22 ^{ab} |
| Kidney functions | Urea (mg/dL) | 37.02±0.88 | 38.19±1.51 | 37.63±1.66 | 120.65±7.46 ^a | 58.5±1.5 ^{ab} | 49.1±1.0 ^b |
| | Creatinine (mg/dL) | 0.21±0.03 | 0.25±0.03 | 0.25±0.03 | 1.82±0.19 ^a | 0.52±0.02 ^b | 0.43±0.01 ^b |

Values were expressed as mean ± standard error, a: significant difference from control group, b: significant difference from toxic group at p<0.000.

Table 6. The effect of *B. variegata* extract before and after incorporating gold nanoparticles against STZ-induced diabetes on hepatic and pancreatic SOD, CAT activities, GSH and TBARS levels.

| | | Control | Extract treated | Nano-treated | Diabetic | D+E treated | D+N treated |
|------------------------|--------------------------|-------------|-----------------|--------------|--------------------------|----------------------------|--------------------------|
| Pancreas tissue | SOD (IU/g tissue) | 49.54±1.74 | 52.11±1.1 | 52.17±0.85 | 13.1±0.81 ^a | 34.3±0.87 ^{ab} | 40.52±0.44 ^{ab} |
| | CAT (IU/g tissue) | 117.23±2.1 | 115.9±1.61 | 115.10±1.39 | 37.26±0.83 ^a | 75.36±1.95 ^{ab} | 92.9±1.67 ^{ab} |
| | GSH (mg/g tissue) | 261±22.91 | 246.33±11.34 | 259.8±10.87 | 79.87±4.29 ^a | 163.12±11.34 ^{ab} | 208.3±5.1 ^b |
| | TBARS (nm/mL) | 50.53±2.25 | 50.1±1.65 | 51.65±1.63 | 184.9±4.1 ^a | 79.1±1.17 ^{ab} | 66.47±0.98 ^{ab} |
| Liver tissue | SOD (IU/g tissue) | 51.27±0.64 | 49.37±0.82 | 49.23±0.83 | 11.57±1.07 ^a | 35.60±0.81 ^{ab} | 40.48±0.62 ^{ab} |
| | CAT (IU/g tissue) | 50.53±1.88 | 48±1.77 | 50.1±0.92 | 7.45±0.57 ^a | 33.98±0.7 ^{ab} | 41.75±1.0 ^{ab} |
| | GSH (mg/g tissue) | 233.69±5.14 | 227.17±5.19 | 227.67±5.03 | 66.33±2.94 ^a | 183.5±2.33 ^{ab} | 201.7±3.65 ^{ab} |
| | TBARS (nm/mL) | 38.51±1.24 | 41.81±1.14 | 40.52±1.55 | 117.43±5.76 ^a | 63.88±1.46 ^{ab} | 52.83±1.1 ^{ab} |

Values were expressed as mean ± standard error, a: significant difference from control group, b: significant difference from toxic group at p<0.000.

liver tissue by seven bands and six from them are considered as common bands and identified at Rfs 0.19, 0.27, 0.39, 0.57, 0.72, and 0.93 (B% 15.16, 14.74, 14.81, 14.80, 14.40, and 12.42; Q% 10.47, 9.80, 10.22, 28.00, 17.31, and 9.52, respectively). No electrophoretic changes noticed in livers of extract and nano-extract treated groups. In liver of diabetic group, it was found that STZ caused alterations represented by hiding the sixth band with one characteristic band was existed at Rf 0.62 (B% 13.87 and Q% 11.27). Therefore, the diabetic group was found with lower SI (85.71%), and hence higher GD (14.29%) as revealed in **Figure 3**. The treatment with *B. variegata* extract and nano-extract showed ameliorative effect against STZ-induced diabetes by hiding the abnormal band with re-appearing the normal one. Therefore,

the SI% and GD restored to normal values (100% and 0%, respectively). It was showed that STZ caused quantitative changes represented by the significant ($p < 0.05$) decrease in quantities of the TP bands. The treatment with the extract could not restore it to normal level. Although the nano-extract could not restore its level to normalcy, it increased the bands quantity significantly ($p < 0.05$) with respect to D and D+E treated groups.

In the pancreas tissue, the native protein pattern was expressed by nine bands and two from them are considered as common bands [Rfs 0.79 and 0.96 (B% 13.50 and 11.36; Q% 11.96 and 13.83, respectively)]. No electrophoretic abnormalities identified in pancreas of extract and nano-extract treated groups. In diabetic group, it was noticed absence of seven normal bands

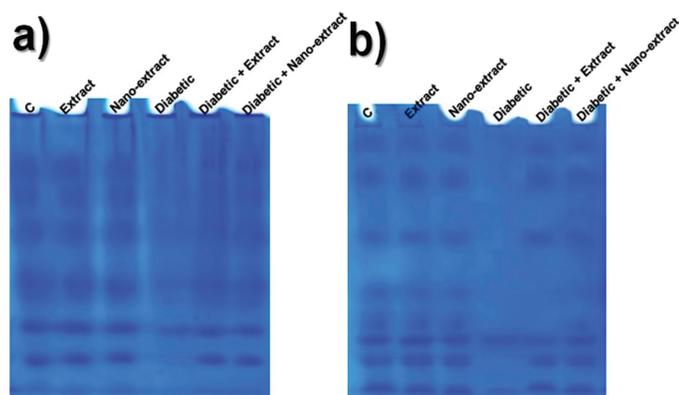


Figure 2. Electrophoretic protein patterns showing the curative effect of *B. variegata* extract before and after incorporating Au-NPs against STZ induced diabetes on (a) liver tissues and (b) pancreatic tissues of rats.

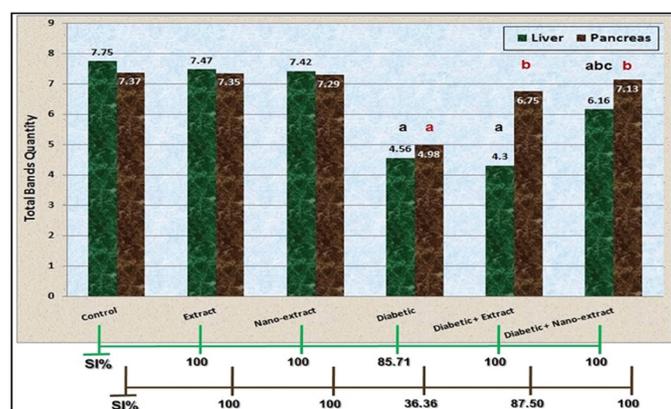


Figure 3. Data showing the ameliorative effect of *B. variegata* extract before and after incorporating Au-NPs against the quantitative and qualitative alterations induced by STZ in protein patterns in liver and pancreatic tissues of rats. Values expressed as mean \pm SE of three replicates, (a) significant difference at $p < 0.05$ from control group, (b) significant difference at $p < 0.05$ from diabetic group, (c) significant difference at $p < 0.05$ from *B. variegata* extract treated group.

without appearance of abnormal ones (Fig. 2b). Therefore, the diabetic group was found with the lowest SI (36.36%), and hence the highest GD (63.64%) (Fig. 3). The extract restored only five normal bands (SI: 87.50% and GD: 12.50%). While the nano-extract showed higher ameliorative effect by restoring all the normal bands (SI: 100% and GD: 0%). Moreover, it was found that STZ caused quantitative changes represented by the significant ($p < 0.05$) decrease in quantities of the TP bands. The treatment with extract and nano-extract increased the bands quantity significantly ($p < 0.05$) comparing to D group.

The electrophoretic lipoprotein pattern in liver tissue of control group was represented by six bands and five from them are considered as common bands identified at Rfs 0.41, 0.59, 0.74, 0.86, and 0.96 (B% 19.35, 19.56, 16.58, 13.33 and 13.63; Q% 13.42, 18.87, 21.99, 6.83 and 8.22, respectively) (Fig. 4a). No changes detected electrophoretically in livers of extract and nano-extract treated groups. It was found that STZ caused alterations represented by hiding the first band without appearance of characteristic bands in diabetic group. Therefore, the diabetic group was found with lower SI (90.91%), and hence higher GD

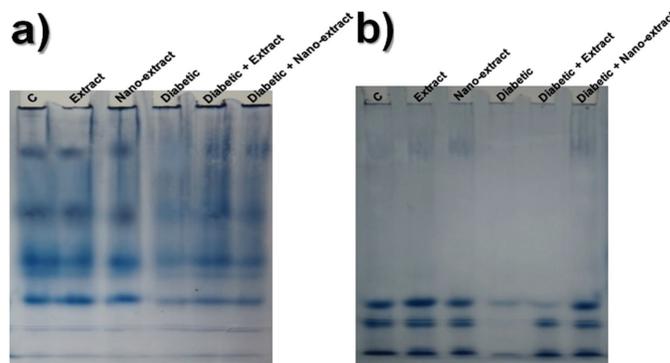


Figure 4. Electrophoretic lipoprotein patterns showing the curative effect of *B. variegata* extract before and after incorporating Au-NPs against STZ induced diabetes on (a) liver tissues and (b) pancreatic tissues of rats.

(9.09%) (Fig. 5). The treatment with extract and nano-extract showed ameliorative effect against STZ-induced diabetes by re-appearing the normal band. Therefore, the SI% and GD restored to normal values (100% and 0%, respectively). Furthermore, data revealed in Figure 5 showed that STZ caused quantitative changes represented by lowering quantities of the total lipoprotein bands significantly ($p < 0.05$). The treatment with extract and nano-extract could not restore it to normal level.

In the pancreas tissue, the native lipoprotein pattern was expressed by four bands and two from them are considered as common bands [Rfs 0.77 and 0.95 (B% 25.75 and 27.27; Q% 25.23 and 23.20, respectively)]. No electrophoretic changes identified in pancreas of extract and nano-extract treated groups. In diabetic group, it was noticed absence of two normal bands without appearance of abnormal ones (Fig. 4b). Therefore, the diabetic group was found with the lowest SI (66.67%), and hence the highest GD (33.33%) as compiled in Figure 5. The treatment with extract restored only one normal band (SI: 85.71% and GD: 14.29%). While the nano-extract showed higher ameliorative effect by restoring the two normal bands (SI: 100% and GD: 0%). In addition, it was showed that STZ caused quantitative changes represented by the significant ($p < 0.05$) decrease in quantities of the total lipoprotein bands. The treatment with extract and nano-extract increased quantities of the total lipoprotein bands significantly ($p < 0.05$) to normalcy as compared to D group.

Native electrophoretic isoenzymes

As illustrated in Figure 6a, the electrophoretic α -amylase isoenzyme was expressed in the healthy liver tissue by two types identified at Rfs 0.23 and 0.73 (B% 50.55 and 49.45; Q% 49.08 and 50.92, respectively). The second type is considered as common band. No electrophoretic changes identified in livers of extract and nano-extract treated groups. In livers of diabetic group, it was found that STZ caused alterations represented by hiding the first type with appearance of two characteristic bands identified at Rf 0.16 and 0.38 (B% 32.28 and 34.86; Q% 26.36 and 36.37, respectively). Therefore, the diabetic group was found with the lowest SI (40.0%), and hence the highest GD (60.0%) as revealed in Figure 7. The treatment with extract restored only one normal type with hiding the abnormal ones (SI: 66.67% and GD: 33.33%). While the nano-extract showed

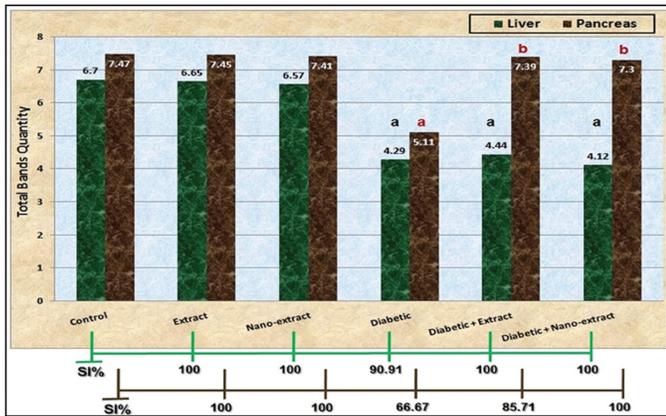


Figure 5. Data showing the ameliorative effect of *B. variegata* extract before and after incorporating Au-NPs against the quantitative and qualitative alterations induced by STZ in lipoprotein patterns in liver and pancreatic tissues of rats. Values expressed as mean ± SE of three replicates, (a) significant difference at $p < 0.05$ from control group, (b) significant difference at $p < 0.05$ from diabetic group.

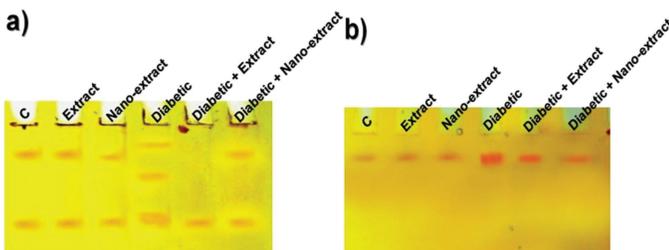


Figure 6. Electrophoretic α -amylase isoenzymes showing the curative effect of *B. variegata* extract before and after incorporating Au-NPs against STZ induced diabetes on (a) liver tissues and (b) pancreatic tissues of rats.

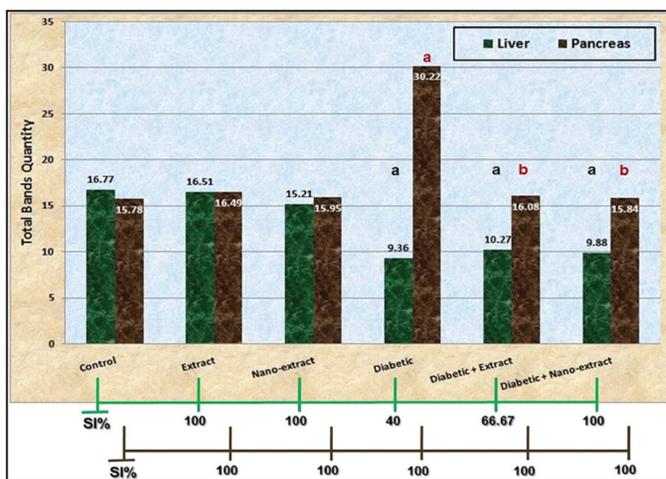


Figure 7. Data showing the ameliorative effect of *B. variegata* extract before and after incorporating Au-NPs against the quantitative and qualitative alterations induced by STZ in α -amylase isoenzymes in liver and pancreatic tissues of rats. Values expressed as mean ± SE of three replicates, (a) significant difference at $p < 0.05$ from control group, (b) significant difference at $p < 0.05$ from diabetic group.

higher ameliorative effect by restoring the two normal bands (SI: 100% and GD: 0%). As regard to the quantities, it was noticed that STZ caused significant ($p < 0.05$) decrease in quantities of

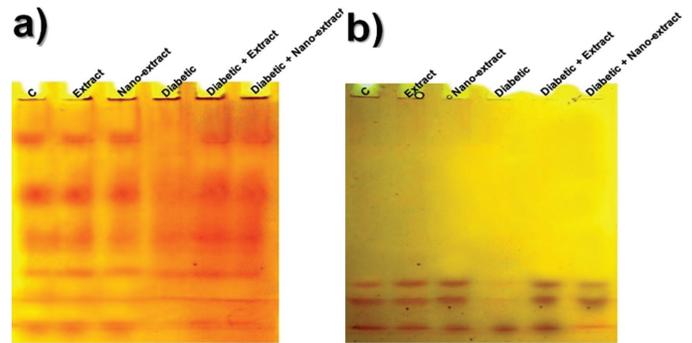


Figure 8. Electrophoretic CAT isoenzymes showing the curative effect of *B. variegata* extract before and after incorporating Au-NPs against STZ induced diabetes on (a) liver tissues and (b) pancreatic tissues of rats.

the α -amylase types as compared to control group. The treatment with extract and nano-extract could not ameliorate these changes. In the pancreas tissue, the electrophoretic α -amylase isoenzyme was represented by only one type identified as common band at Rfs 0.21 (B% 100 ; Q% 100, respectively) (Fig. 6b). In pancreas of diabetic group, it was found that STZ caused no alterations at qualitative level but it caused changes at quantitative level through increasing quantities of the α -amylase band significantly ($p < 0.05$) as compared to control group (Fig. 7). The treatment with extract and nano-extract restored quantities of the α -amylase types to normal level.

In control liver, it was noticed that CAT isoenzyme was represented electrophoretically by seven types and six from them are considered as common bands and identified at Rfs 0.39, 0.57, 0.71, 0.82, 0.92 and 0.932 (B% 19.45, 14.97, 10.23, 10.10, 11.37 and 18.83 ; Q% 44.23, 17.24, 8.05, 8.52, 3.52 and 5.29, respectively) (Fig. 8a). In livers of diabetic group, it was found that STZ caused alterations represented by hiding the first type without appearance of abnormal ones. Therefore, the diabetic group was found with lower SI (92.31%) and higher GD (7.69%) as compiled in Figure 9. The treatment with extract and nano-extract showed restored the normal CAT type that disappeared by STZ. Therefore, the SI% and GD restored to normal values (100% and 0%, respectively). It was showed that STZ caused quantitative changes represented by the significant ($p < 0.05$) decrease in quantities of the total CAT bands. The treatment with extract and nano-extract could not restore it to normal level but they significantly ($p < 0.05$) increased the bands quantity comparing to D group. The nano-extract showed higher ameliorative effect noticed by increasing the bands quantity significantly ($p < 0.05$) as compared to diabetic group treated with extract.

In the pancreas tissue, the CAT isoenzyme was expressed by four bands and two from them are considered as common bands [Rfs 0.94 and 0.95 (B% 31.00 and 24.73; Q% 18.04 and 18.23, respectively)]. No deviation in CAT isoenzyme in pancreas of extract and nano-extract treated groups from control. In diabetic group, it was found that STZ caused absence of the first and second CAT types without appearance of abnormal bands (Fig. 8b). Therefore, the diabetic group was found with lower SI (66.67%) and higher GD (33.33%) as documented in Figure 9. The treatment with extract and nano-extract restored the normal CAT types, and hence restored SI and GD to normal values (SI: 100% and GD: 0%). In addition, it was found that STZ caused

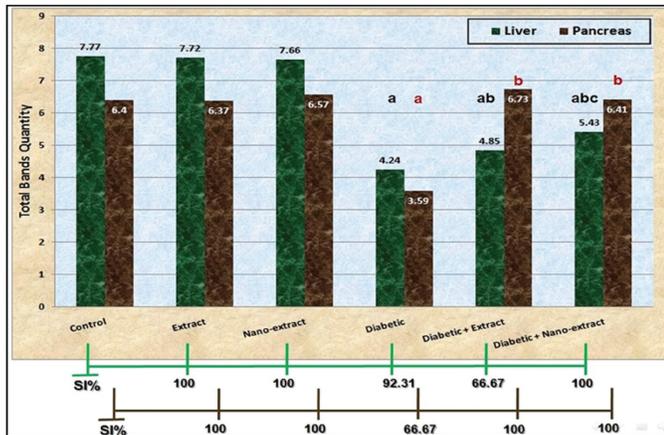


Figure 9. Data showing the ameliorative effect of *B. variegata* extract before and after incorporating Au-NPs against the quantitative and qualitative alterations induced by STZ in CAT isoenzymes in liver and pancreatic tissues of rats. Values expressed as mean \pm SE of three replicates, (a) significant difference at $p < 0.05$ from control group, (b) significant difference at $p < 0.05$ from diabetic group, (c) significant difference at $p < 0.05$ from *B. variegata* extract treated group.

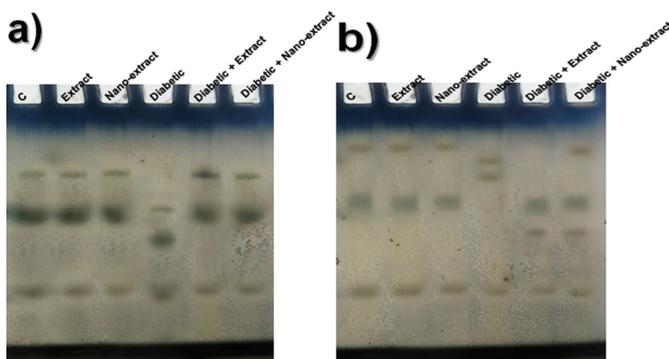


Figure 10. Electrophoretic POX isoenzymes showing the curative effect of *B. variegata* extract before and after incorporating Au-NPs against STZ induced diabetes on (a) liver tissues and (b) pancreatic tissues of rats.

quantitative changes represented by decreasing quantity of the total CAT bands significantly ($p < 0.05$). The extract and nano-extract significantly ($p < 0.05$) increased quantities of the CAT bands comparing to D group. The nano-extract showed higher ameliorative effect through significantly ($p < 0.05$) increasing the bands quantity as compared to D group treated with extract and restored its level to normal values.

As presented in Figure 10a, the native POX isoenzyme was represented electrophoretically in healthy liver tissue by three types and only one of them is considered as common band (Rf 0.77, B% 33.52, and Q% 32.88). No electrophoretic abnormalities noticed in livers of extract and nano-extract treated groups. In livers of diabetic group, it was found that STZ caused qualitative alterations represented by hiding the first and second POX types with existence of two characteristic bands identified at Rfs 0.43 and 0.55 (B% 28.72 and 36.04 ; Q% 21.12 and 50.97, respectively). Therefore, the diabetic group was noticed with the lowest SI (33.33%), and hence the highest GD% (66.67%) as illustrated in Figure 11. The treatment with extract and nano-extract exhibited beneficial effect against STZ-induced diabetes by hiding the

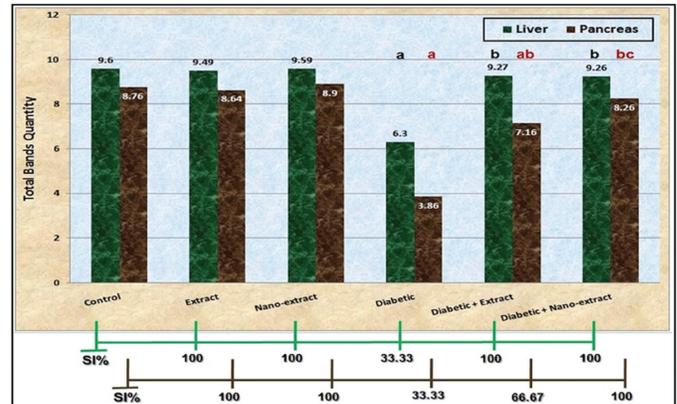


Figure 11. Data showing the ameliorative effect of *B. variegata* extract before and after incorporating Au-NPs against the quantitative and qualitative alterations induced by STZ in POX isoenzymes in liver and pancreatic tissues of rats. Values expressed as mean \pm SE of three replicates, (a) significant difference at $p < 0.05$ from control group, (b) significant difference at $p < 0.05$ from diabetic group, (c) significant difference at $p < 0.05$ from *B. variegata* extract treated group.

abnormal bands associated with re-appearing the normal ones. Therefore, the SI% and GD restored to normal values (100% and 0%, respectively). It was found that STZ caused quantitative changes represented by the significant ($p < 0.05$) decrease in quantities of the total POX bands. The treatment with extract and nano-extract restored the bands quantities to normal level.

In the pancreas tissue, POX isoenzyme was expressed by three types. The third type is considered as common band [Rfs 0.78 (B% 31.49 and Q% 41.51)]. No deviation in POX isoenzyme in pancreas of extract and nano-extract treated groups from control. STZ caused absence of the first and second POX types with appearance of two abnormal bands identified at Rfs 0.24 and 0.31 (B% 33.75 and 32.48; Q% 28.99 and 37.86, respectively) (Fig. 10b). Therefore, the diabetic group was found with the lowest SI (33.33%), and hence the highest GD (66.67%) as presented Figure 11. The treatment with extract restored only one normal type with hiding one abnormal band (SI: 66.67% and GD: 33.33%). While the nano-extract exhibited a higher ameliorative effect by restoring the two normal bands (SI: 85.71% and GD: 14.29%). Moreover, STZ caused quantitative changes represented by lowering quantities of the total POX bands significantly ($p < 0.05$). The extract and nano-extract significantly ($p < 0.05$) increased quantities of the total bands as compared to D group. The nano-extract showed higher ameliorative effect noticed by increasing the bands quantity significantly ($p < 0.05$) comparing to D+E and restored the bands quantities to normal values.

Histopathological study

As presented in Figure 12, it was noticed that diabetic group appeared with fibrosis associated with inflammatory cells infiltration, congestion in the portal area, and dilatation in central vein. This was in accordance with Teoh *et al.* (2009) who postulated that involvement of the hepatocytes with features of inflammation in diabetic rats. The treatment with *B. variegata* extract and *B. variegata*- gold nano-extract ameliorate the histopathological changes, especially in the diabetic group that treated with nano-extract where it appeared with normal architecture like control.

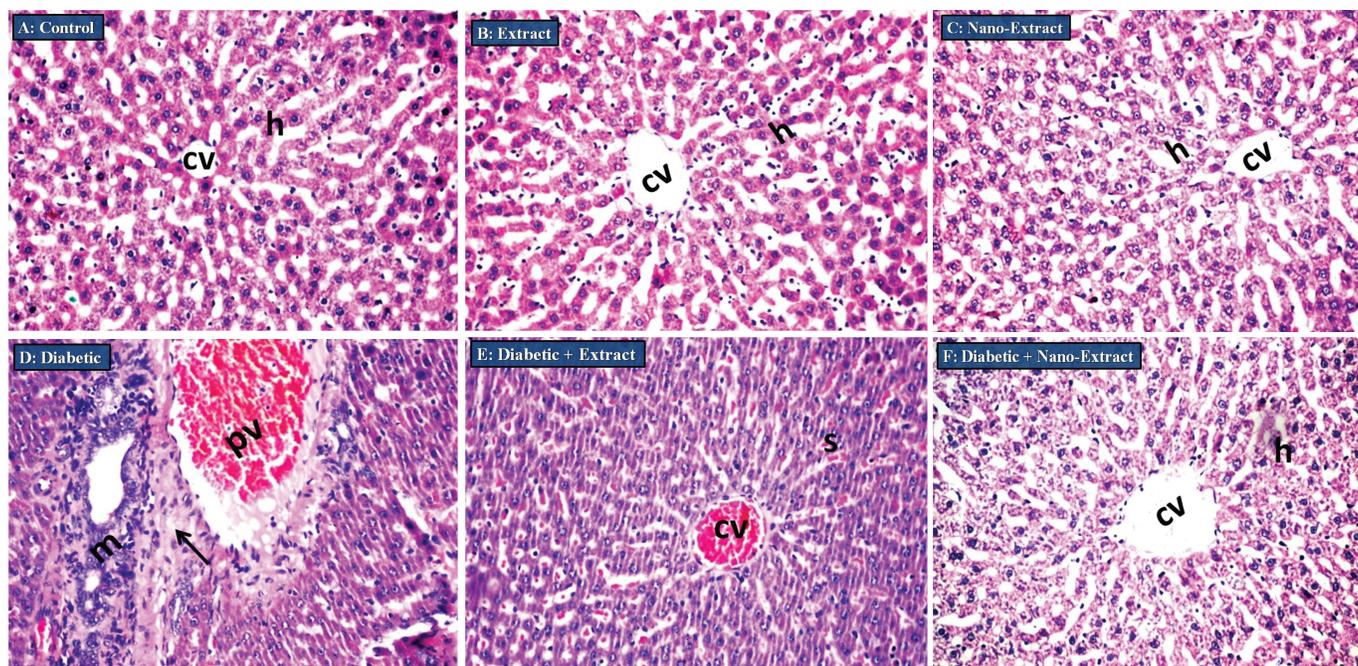


Figure 12. (A) Section of liver of control group showed normal architecture of hepatocytes (h), (B) Section of liver of extract treated group showed normal hepatic lobule and hepatocytes, (C) Section of liver of nano-extract treated group showed no histopathological changes as in control group, (D) Section of liver of diabetic group showed fibrosis in the portal area (arrow) with inflammatory cells infiltration (m) and congestion in the portal vein (pv), as well as, dilatation and congestion in the central veins (cv), (E) Section of liver of diabetic group treated with extract showed mild congestion in the central vein and sinusoids, (F) Section of liver of diabetic group treated with nano-extract showed no histopathological alteration in hepatic architecture as in control group (H&E \times 16).

The histopathological analysis of pancreatic tissue showed that there was no histopathological alteration in control group where the islets of Langerhans as endocrine portion as well as the acini and ducts system as exocrine one were recorded. The un-toxic effect of ethanolic extract and gold nano-extract were confirmed when compared both groups with control group where, no histological alterations were detected (Fig. 13).

It was showed that either complete absence of islets of Langerhans cells in some diabetic samples or atrophy in most of islands of Langerhans cells all over the lobules and only few islets cells were intact in other diabetic samples (Fig. 13). The treatment with both ethanolic extract and gold nano-extract restored the normal structure of pancreatic β -cells indicating the protective effect of both of them on pancreatic tissue.

DISCUSSION

The available synthetic antidiabetic drugs (insulin and other oral anti-diabetic agents) were associated with side effects, such as hypoglycemia and diarrhea in addition to failure of liver and kidney (Zhang *et al.*, 2016). It was necessary to study the potential benefits of different plant extracts for diabetes management (Ogunyinka *et al.*, 2015). Nanotechnology is emerged as a new strategy that used to increase the efficacy and bioavailability of medicinal plants (Gunasekaran *et al.*, 2014). Recently, scientists have come to be increasingly interested in preparation of Au-NPs, owing to their special physical and chemical properties in extensive areas like catalysis, bio-labeling, drug delivery, and packaging applications (Daniel and Astruc, 2004; Pal *et al.*, 2013; Youssef and El-Sayed, 2018). Our study aimed to evaluate the effect of the integration of Au-

NPs in *B. variegata* extract on its antidiabetic, antioxidant, and hypolipidemic effect in DM induced rats.

During the present study, it was found that ethanolic *B. variegata* leaves extract showed the highest concentration of total polyphenols, total antioxidant capacity, iron reducing power, and scavenging activity against free radicals attack as compared to the other plant extracts. Our results were in accordance with Kalim and Nikalje (2017) who supported that the ethanolic extract of this plant exhibited high scavenging activity against both Reactive Oxygen (ROS) and Nitrogen Species (RNS). This might be attributed to presence of the active phenolic compounds, including quercetin, naringenin, rutin, and luteolin that exhibit redox properties letting them to act as hydrogen donors and reducing agents (Abdel-Aziz *et al.*, 2014). In addition, Mishra *et al.* (2013) reported that the various *B. variegata* leaves extracts possess potent capability to combat the oxidative stress due to its iron binding, radical neutralization and reducing power ability.

During the current study, it was found that *B. variegata*-gold nano-extract showed higher concentration of active components (total phenolic compounds and total flavonoids). This was in agreement with Alegria *et al.* (2018) who reported that amount of the polyphenols increased in the extract during the preparation of nano-extract indicating their effective involvement in nanoparticles synthesis. These findings were supported by many recent studies, which explained that the integration of metal nanoparticles into the plant extract increased the active phyto-constituents. Consequently, this leads to elevating its total antioxidant ability, iron reducing power, and hence increasing the free radicals scavenging ability as compared to the plant extract alone (Abdelhady and Badr, 2016; Aboulthana *et al.*, 2019).

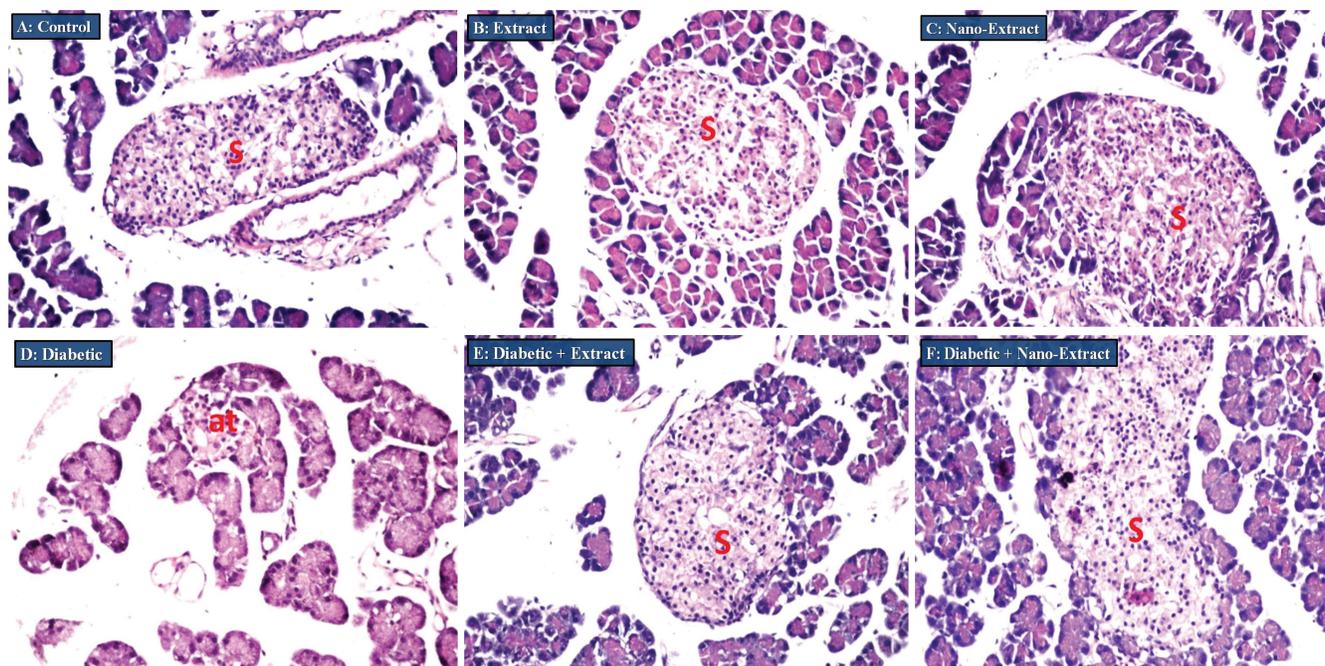


Figure 13. (A) Section of pancreas of control group showing normal histological structure of islands of Langerhans cells as endocrine portion as well as the acini with duct system as exocrine one (H&E \times 40), (B) Section of pancreas of extract treated group, (C) Section of pancreas of nano-extract treated group showed normal histological structure of islands of Langerhans and no histopathological changes as in control group, (D) Section of pancreas of diabetic group showing atrophy (at) in most of islands of Langerhans cells all over the lobules. In other field, it showed intact few islands of Langerhans cells or complete absence of the islands of Langerhans cells in the lobules, (E) Section of pancreas of diabetic group treated with extract and (F) diabetic group treated with nano-extract showed no histopathological alterations in pancreatic architecture as in control group (H&E \times 40).

Our results showed that body weight was decreased in D group with respect to control group. This might be due to the inability for utilizing carbohydrates to get energy because of the deficient in insulin secretion. Therefore, the body directed to get energy through degradation of fats and muscles (proteins) as reported by Choudhary *et al.* (2014). While the improvement in body weight after treatment with both the extract and nano-extract indicated the improvement in insulin secretion, which was in agreement with the results that obtained by Hassan *et al.* (2015) and supported by Kulkarni and Garud (2016).

The present study showed a significant elevation in blood glucose associated with a decreasing in insulin level in diabetic rats as compared to control group. This attributed to the effect of STZ, which lead to destruction of β cells of pancreatic islets and then reduction in insulin release. This was in accordance with Roder *et al.* (2016) who mentioned that the reduction in insulin secretion decreased glucose entry into tissues, increased glycogen breakdown, gluconeogenesis and glucose production by liver, and finally increased blood glucose levels.

Levels of glucose decreased and insulin increased after treatment with both extract and nano-extract, which might be due to the presence of polyphenols in our extract, which enhance glucose uptake. In accordance with our results, Naowaboot *et al.* (2012) and Mohan *et al.* (2013) showed that the presence of different active components as tannins and quercetin improve the efficacy of *B. variegata* extract to restore the integrity of β - cells of pancreas and hence upturn glucose uptake and metabolism. Moreover, Frankish *et al.* (2010) and Kumar *et al.* (2012) reported that *B. variegata* extract was found to activate insulin secretion

in insulin secreting cell line. In addition, Rashid (2014) added that *B. variegata* leaves contain insulin-like protein, which might be responsible for its antidiabetic effect, where plant and animal insulin share metabolic functions as carbohydrate metabolism regulation, protein phosphorylation, and transporting glucose into tissues.

The α -amylase is a digestive enzyme found mainly in saliva and pancreatic juice. It hydrolyses α -(1,4)-D-glycosidic linkages of dietary carbohydrates as starch and other glucose polymers to oligosaccharides and disaccharides, which then hydrolyzed to monosaccharides and absorbed through the small intestine (Najafian *et al.*, 2011). The current study showed that STZ caused significant decrease in the α -amylase quantity in livers of diabetic rats. This was in accordance with Mufeed *et al.* (2014) and supported by Udia *et al.* (2016) who reported that the enzyme quantity decreased due to the hormonal and metabolic alterations sequel to diabetes. Moreover, STZ caused alterations at qualitative level through changing the number and arrangement of the bands and hence leads to lowering the SI%. Otherwise, it was observed that STZ caused a significant increase in quantities of the α -amylase bands and it was represented by only one thick band in pancreas of diabetic rats. It was reported that the enzyme quantity increased due to the abnormal effectiveness of the acinar pancreatic cells to biosynthesize this enzyme or its effective role in glucose hydrolysis (Kamarýt *et al.*, 1993; Aboulthana *et al.*, 2018). The treatment with extract before and after incorporating Au-NPs ameliorated the qualitative and quantitative abnormalities in the electrophoretic α -amylase pattern. Azevedo *et al.* (2006) attributed this amelioration effect of *B. variegata* leaves extract to

its antidiabetic effect, which is related to the presence of insulin-like proteins. In addition, the presence of active constituents as phenolic compounds inhibits α -amylase activity due to their free radical scavenging and antioxidant activities (Rao and Mohan, 2017).

Diabetes is linked to hyperglyceridemia and hypercholesterolemia, which represents high risk factors for atherosclerotic disease (Andallu *et al.*, 2009). Insufficient insulin secretion leads to inactivation of lipoprotein lipase, which is responsible for TGs hydrolysis, which consequently leads to increasing in TGs levels. The TGs increases secretion of LDL-c and decreases HDL-c. In the present work, it was found that levels of TGs, TC, LDL-c were significantly increased associated with a decreasing in HDL-c in D group as compared to control group, while treatment with both *B. variegata* extract and gold nano-extract decreased TGs, TC, LDL-c levels, and increased HDL-c. This might be due to presence of the various phenolic compounds in our extract. In accordance of our results, Dhanya *et al.* (2014) mentioned that rutin and quercetin were responsible for enhancing glucose uptake.

The results showed that serum TP level was decreased and the electrophoretic protein and lipoprotein patterns was found to be altered in diabetic group as compared to control group which may be due to the action of STZ which causes damage of proteins by free radicals induction. In accordance with our results, Karthikesan *et al.* (2010) and Bhargavi *et al.* (2015) explained that the decline in TP was due to the elevation of glucose level, which leads to glycosylation of proteins and lipoproteins through the reaction of glucose with the protein portions. Furthermore, Blasiak *et al.* (2004) and Aboulthana *et al.* (2018) postulated that these electrophoretic changes may occur because of the reduction of the antioxidant activities, and hence accumulation of oxidative stress products.

The treatment with gold *B. variegata* nano-extract decreased the qualitative and quantitative disturbances induced by STZ on the electrophoretic protein, lipoprotein patterns, and serum TP level more than *B. variegata* extract itself as compared to diabetic group. This might be attributed to the presence of various active phyto-constituents that increased after incorporating Au-NPs as supported during the current study. The presence of the polyphenolic compounds in the extract exhibited antihyperglycemic effect by reducing the blood glucose level, and hence the alterations that occurred as a result of the glycosylation process might be decreased by the extract (Shanmugasundaram *et al.*, 1990). The Au-NPs incorporated into the extract are carried across the cell membrane as they are very small in size and have a prolonged effect in biological systems (Govindaraju *et al.*, 2009).

Liver is considered as one of the central metabolic organs, which play a vital role in regulating and maintaining homeostasis in the body. Diabetes is closely linked to structural and functional liver abnormalities as well as impairment of the pancreas. The changes in target proteins and lipid metabolism as well as in the antioxidant status in liver are the most common abnormalities that are usually associated with diabetes (Saxena *et al.*, 1993; Zeindl-Eberhart *et al.*, 2001).

Results of the current study showed that AST, ALT, and ALP activities were significantly elevated in D group comparing to the control group. This increase might be due to the cellular damage

that caused by STZ which causes leakage of liver enzymes into the blood stream. These findings agreed with Zafar *et al.* (2009) and Kasetti *et al.* (2010) who documented that STZ causes liver injury leading to release of the enzymes, and hence increased their levels in the blood. There was a significant decrease in liver enzymes in D+E and D+N treated groups comparing to D group, indicating the hepato-protective effect of ethanolic *B. variegata* extract and nano-extract. The un-toxic effect of the ethanolic extract or the nano-extract on the animals was noticed as liver enzymes in E and N treated groups as in control group.

Increased urea and creatinine in diabetes indicating impairment of kidney functions (Ronco *et al.*, 2010). Our results showed an increment in urea and creatinine levels in STZ-induced diabetic animals as compared to control group. In agreement with our results Hassan *et al.* (2015) showed that the elevation of urea and creatinine might be due to increased protein catabolism. Ethanolic extract and gold nano-extract of *B. variegata* attenuate the levels of urea and creatinine comparing to diabetic group, which may be due to the antioxidant properties of the extract.

When the balance between ROS generation and ROS scavenging systems is lost, oxidative stress was evolved. The continuous free radicals production that associated with hyperglycemia will eventually cause cellular damage or dysfunction as lipid peroxidation, protein glycation, and glucose autoxidation. Enzymatic and non-enzymatic antioxidants are responsible for the protection of the body against free radicals attack by converting them to less toxic compounds (Ahmed, 2005; Mohan *et al.*, 2013). The present study showed that hepatic and pancreatic antioxidant enzyme activities (CAT, SOD), and GSH level were decreased and lipid peroxidation was increased in D group as compared to control group which due to the induction of free radicals by STZ. Our results were in agreement with Rahman (2007) who mentioned that the enzymatic and non-enzymatic antioxidants were decreased in DM because of their consumption to attenuate oxidative stress. Furthermore, STZ caused alterations in the different electrophoretic CAT and POX isoenzymes in both of liver and pancreas of diabetic rats. This was in agreement with Oyenih *et al.* (2015) and Chandirasegaran *et al.* (2018). This might be due to degeneration of protein contents or changes in the metabolic pathways because of free radicals attack in addition to the glycation process, which inhibits activity of these enzymes (Ramanathan *et al.*, 1999; Al-Enazi, 2014).

Treatment of diabetic rats with either extract or gold nano-extract of *B. variegata* improved the antioxidant defense system through increasing levels of antioxidants, decreasing TBARS (lipid peroxidation product) level and ameliorating the alterations in electrophoretic CAT and POX isoenzymes in both liver and pancreas as compared to diabetic group. This might be due to presence of the active phyto-constituents that exhibited antioxidant properties and increased after incorporating nanoparticles. Different studies suggested that antioxidant defense mechanisms in pancreatic islets could protect from diabetes. It was explained that *B. variegata* has a free radical scavenging activity due to its phenolic contents as quercetin, rutin, and kaempferol (Rashid, 2014; Sheweita *et al.*, 2016; Kalim and Nikalje, 2017). Dixon and Palva (1995) explained that these phenolic compounds are the first line of a complex defense system against different stressors.

Our biochemical and electrophoretic studies were supported by the histopathological investigation which revealed that the treatment with both extract and nano-extract ameliorated the deleterious alterations that induced by STZ in hepatic and pancreatic tissues.

CONCLUSION

The incorporation of Au-NPs into the *B. variegata* extract improved its efficacy, where its polyphenolic contents, total antioxidant activity, iron reducing power, and scavenging activity against free radicals attack were increased as compared to extract only. Therefore, the *B. variegata* gold nano-extract exerted more efficient anti-diabetic effect through restoring the normal architecture of pancreatic β -cells in addition to the antioxidant and hypolipidemic activity against STZ-induced diabetes in rats than extract alone.

DECLARATIONS

Author contribution

Abeer Hamed Abdel-Halim and Mamdouh Mouad Ali Hassan proposed the research concept. Abeer Hamed Abdel-Halim and Amal Abdel-Azeem Fyiad performed the extraction and designed the experimental model. Wael Mahmoud Aboulthana collected plant samples, performed phytochemistry studies, and helped in analyzing data. Abeer Hamed Abdel-Halim, Amal Abdel-Azeem Fyiad, Wael Mahmoud Aboulthana, and Nermin Mohammed El-Sammad performed the experimental work and provided reagents/materials necessary for experiments. Amal Abdel-Azeem Fyiad and Wael Mahmoud Aboulthana helped in writing the manuscript

Ahmed Mahmoud Youssef was responsible for the preparation of the gold nano-extract and analyzing its data.

Abeer Hamed Abdel-Halim interpreted, analyzed the data, wrote, and correspond the manuscript.

All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Additional information

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