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Research article

Bioactivity and antidiabetic properties of *Malva parviflora* L. leaves extract and its nano-formulation in streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes is a drastic health problem resulting from an endocrine disorder. *M. parviflora* L. might represent an antioxidant-rich food source and thus applies to pharmaceutical and therapeutic applications in oxidative stress-related degenerative diseases. In the current work, we assessed the antidiabetic activity of *M. parviflora* L. leaves extract and its nano-formulation in rats. *M. parviflora* L. bioactivity was evaluated by both antioxidant and antimicrobial assays. The nano-formulation characteristics (Mass, TEM, and Zeta potential) were evaluated. Twenty-four male Sprague-Dawley rats were administered streptozotocin (STZ) intraperitoneally for only one dose (35 mg/kg body weight). All of the nutritional and biochemical parameters were statistically analyzed. The results showed that *M. parviflora* L. is rich in phenolics and flavonoids with high antioxidant action. The antifungal activity of the extract was evident, especially with *Fusarium culmorum and aspergillus flavus*. The extract and its nano-formulation have shown antidiabetic properties when tested on diabetic rats as they improved all the biochemical parameters; decreased glucose level in serum, increased insulin production, marked improvement in lipid profile, liver and kidney functions, and that was more proved with the histopathological examinations. Conclusively, *M. parviflora* L. extract and its nano-formulation could attenuate or effectively help in controlling diabetes through its therapeutic properties exhibited by the action of the plant antioxidant components.

1. Introduction

Diabetes is a drastic health problem resulting from an endocrine disorder. Its severity is not only due to the many consequences but also to its fast-developing rate around the globe. The World Health Organization (WHO) estimated that the number of diabetic patients by 2025 will reach about 300 million (Abu-Odeh and Talib, 2021). The Middle Eastern countries are considered the fastest growing regions in this diabetic increase rate as, according to statistics, it will almost double by 2035, representing an increase in social and economic burden (Mannan et al., 2014; Abuyassin and Laher, 2016; Sathasivampillai et al., 2017). Diabetes is also considered a metabolic disorder with disrupted digestion and nutrient absorption, attributable to an abnormality either in insulin secretion or action, or even both, that leads to persistent hyperglycemia and several consequences, including the distress of many vital organs (Mannan et al., 2014; Abuyassin and Laher, 2016; Sathasivampillai et al., 2017).

Dietary antioxidants have been hypothesized to have a protective effect against the development of diabetes by inhibiting peroxidation chain reactions (Halliwell and Gutteridge, 2015). It seems plausible that a sufficient intake of antioxidants plays an important role in protection against type 2 diabetes. However, little epidemiological evidence is available on the role of dietary antioxidant intake in prevention of type 2 diabetes.

What is more, most of the prescribed hypoglycemic drugs or even insulin, are associated with unwanted side effects. Therefore there is an increasing demand of natural products with anti-diabetic activity and less or none unwanted side effects. Herbal medicines are a good option because of their comparably therapeutic effects and nontoxic side effects. As they are derived from the plant has been used to cure various illnesses,

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including diabetes. Different parts of more than 600 plant species are traditionally used in treating diabetes, where the determining factors are mainly availability and safety (Verma et al., 2018). Little mallow (*Malva parviflora* L.) is a perennial family Malvaceae and genus *Malva*, having the common name 'mallow' that includes about 25–40 species of herbs, including biennial, annual, and perennial plants. *Malva parviflora* (known as cheese weed) is growing in waste ground, roadsides and desert plains in Egypt (Zohary, 1987; Ridh et al., 2018). This genus is native to the tropical, temperate, and subtropical regions of Asia, Africa, and Europe. *M. parviflora* might represent an excellent antioxidant-rich food and thus be used in pharmaceutical and therapeutic applications in degenerative disorders linked to oxidative stress (Farhan et al., 2012). Antioxidants in the plant's phenolic components impede the oxidation of low-density lipoproteins, platelets, aggregation as well as the destruction of red blood cells (Cheynier, 2005).

Up till 2012, a survey of the literature revealed that no studies on the potential hypoglycemic effects of this plant had ever been made yet till Perez et al., (2015) reported the outstanding attributes of *Malva Parviflora against diabetes as M. parviflora* leaves can efficiently inhibit insulin resistance, lipid abnormalities and oxidative stress, indicating that its therapeutic properties may be due to the interaction plant components and a further study by (Gutiérrez, 2017) reported also that oleanolic acid derivative from *Malva parviflora* had hypolipidemic and hypoglycemic, anti-inflammatory, activities, improve insulin resistance and hepatic enzymes in streptozotocin-induced diabetic mice.

Nanotechnology has the potential to boost conventional medicine by aiding in the discovery, creation & distribution of a variety of assistance techniques to promote health and minimize the consequences of a variety of illnesses which is being done to improve the technologies that can be used or are being adjusted for traditional medical research. By showcasing these technologies, it is anticipated that the potential for nanomaterials to change conventional medical research will be acknowledged (Abou Baker & Mohammed, 2022).

Thus, there is an excellent need for scientific studies on the characteristics of these traditional remedies obtained from natural sources. There's no studies were done before on the *Malva parviflora* L. by using nanotechnology. So, this novel study aimed to evaluate *Malva* extract's bioactivity and its nano-formulation characteristics as an innovative method in diabetes treatment. As *Malva parviflora* L. leaves extract nanoformulation provide cost effectiveness, high efficiency and lower amount of the extract used that could attenuate or effectively help in controlling diabetes via *in vitro* and *in vivo* and provide more safety profile than the normal extract.

2. Materials and methods

2.1. Materials

Misr for Food Additives (MISAD), Giza, Egypt, provided the emulsifier mono and diglyceride 60%, while in Mumbai, India, the pectin was supplied by Sisco Research Laboratories (SRL). Commercial quality granulated cane sugar had been acquired at the local store. It was manufactured by Sugar and Integrated Industries. The rest of the chemicals and solvents had been acquired from the company in Hawamdia. MERCK, USA.

2.2. Methods

2.2.1. Plant material and preparation

Malva parviflora L. leaves were gathered from roadside vegetation in Banha, province of Kalubya, Egypt, in June 2021. It was in the blossoming stage, and seeds were purchased from the local market. The leaves were dried in the shade until they reached a moisture content of 10%. In contrast, for storage, the seeds were dried in the sun, ground up mechanically, and afterward stored in a vacuum desiccator till required after being adequately cleaned to eliminate any unwanted elements besides other seeds, stones, or short stalks.

2.2.2. Extraction of phenolic compounds

Malva parviflora L. leaves, powdered, weighing 500 g, were extracted using hot distilled water at room temperature. A rotary evaporator evaporated the aqueous extract.

2.2.3. Identification of phenolics and flavonoids using HPLC

By using an Agilent 1260 series HPLC, our hypotheses were confirmed. The separation was accomplished using an Eclipse C18 column (4.6 mm × 250 mm i. d., 5 μm). Water (A) and 0.05 percent trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 ml/min made up the mobile phase. The linear gradient had been sequentially programmed into the mobile phase as described in the following: 0 min (82% A), 0–5 min (80% A), 5–8 min (60% A), 8–12 min (85% A), 12–15 min (85% A), and 16–18 min (82% A). At 280 nm, the multi-wavelength detector was monitored. Injection volumes for each of the sample solutions were 10 μl. 35 °C was maintained as the column temperature.

2.2.4. Determination of total phenol (TP), total flavonoid (TF) contents, and antioxidant activity of Malva parviflora L. Leaves

The TP content of the *Malva parviflora* L. leaves was evaluated using the Folin-Ciocalteu reagent based on (Singleton and Rossi, 1965) technique, with gallic acid as standard. The aluminum chloride colorimetric technique measured the TF content (Willet et al., 2002). To create the calibration curve, catechin was utilized. DPPH, a measure of free radical scavenging ability, was formed by Hwang & Do, 2014).

2.2.5. Nano-formulation preparation of Malva parviflora L

A nano-formulation was prepared as a nano-formulation using the solvent emulsification-diffusion process of creative modifications (Ezzat et al., 2017). Transmission Electron Microscopy and the Zeta Sizer Nano-ZS were used to evaluate nano-formulation. This study has an under-reviewed patent by the Egyptian Patent Office, Academy of Scientific Research & Technology, covering the nano preparation process and the relevant measurements data. The patent number is 1956/2020.

2.2.6. Nanoparticle measurement techniques

2.2.6.1. Mass spectrum (MS). JEOL (JMS-AX500) mass spectrometry was analyzed at the Central Lab of the National Research Centre to assess the *Malva parviflora* L. graph.

2.2.6.2. Transmission electron microscopy (TEM). The samples, which were preserved on a carbonized-copper grid, were evaluated using a TEM (JEM-1234) that operates at a 120 kV voltage, 600,000 x magnification power, 0.3 nm resolving power, a CCD camera, and a temperature control system with a range of -190 °C–1000 °C.

2.2.6.3. Zeta Sizer Nano ZS. The extract of *Malva parviflora* L. was assayed for size distribution (by number), polydispersity index (PDI), and ζ-potential via photon correlation spectroscopy at 25 °C by the use of a Nano Zeta Sizer ZS (Malvern Instruments Inc., Southborough, MA).

2.2.7. Antimicrobial assays

2.2.7.1. The tested microorganisms' strains of bacteria and fungi. All the toxigenic fungal strains under investigation were gained via the Agro-food microbial culture collection at the Institute of Sciences of Food Production (ISPA), Italy. The strains of fungi included *Aspergillus flavus ITEM 698, Aspergillus parasitics ITEM 11, Penicillium chrysogenum ATCC 48271*, and *Fusarium culmorum KF846.*

2.2.7.2. Microorganisms' growth media. To reactivate fungal strains, potato dextrose agar (PDA) and Sabouraud Dextrose agar (SDA) were used for culture. The fungal strains were reactivated using the SDA media with chloramphenicol. And then, it was maintained using PDA media with chloramphenicol.

2.2.7.3. Preparation of the conidial suspension of fungi. A sterile 0.01 % aqueous Tween 80 solution was applied to culture plates. The plate surface was scraped with a bent glass rod to encourage the release of conidia, allowing conidia to be collected from cultures that had been in existence for seven days. Using a Burker-Turk counting chamber (Heamocytometer), the conidia concentration was raised to 106 conidia/mL.

2.2.7.4. Determination of antifungal activity. Antimicrobial activity was assessed using an agar well diffusion assay (Sabry et al., 2021). The test susceptibility was calculated by measuring the inhibition zone diameter around the well.

2.2.8. The biological evaluation of experimental animals

2.2.8.1. Animals. Twenty-four male Sprague-Dawley rats between the ages of 1 and 2 months weighing 200–220 g were kept constant on a pivotal synthesized diet and water over seven days prior to the experiment to allow for adaptation and typical growth and behavior. To acclimatize the rats, solid-bottom cages were employed in a temperature-controlled (23 °C), 40–60g/100g absolute moisture, and light (12 h dark/light cycle) environment free of chemical contamination. The Egyptian National Research Centre's Ethical Committee of Medical Research authorized the animal experiment (Approval no. 19–522) in compliance with the UK's Animals (Scientific Procedures) Act, 1986 and accompanying recommendations, as well as EU Directive 2010/63/EU for animal studies (Publication No. 85–23, revised 1985).

2.2.8.2. Induction of diabetes. A single dosage of streptozotocin (STZ) (35 mg/kg body weight) dissolved in 50 mM of cold citrate buffer (pH 4.5) was used to induce diabetes (Mishra et al., 2019). STZ was given intraperitoneally on the first day of the trial, and blood glucose levels assessed after two days revealed that the rats had diabetes.

2.2.8.3. Diet composition. The basic synthesized diet included casein (150 g/kg diet), unsaturated fat (100 g/1 kg diet), sucrose (220 g/kg diet), maize starch (440 g/kg diet), cellulose (40 g/kg diet), salt mixture (40 g/kg diet), and vitamin mixture (10 g/kg diet) (AOAC, 1990; Ezzat et al., 2017). The AIN-93M diet created the salt and vitamin mixtures (Reeves, 1993).

2.2.8.4. Experimental design. A total of 24 rats will be split into four groups of six each:

- **Negative control:** Healthy rats will be fed a synthetic base diet.
- **Diabetic control (positive):** STZ-induced Diabetic rats were fed a synthetic base diet.
- **Group (1):** STZ-induced diabetic rats fed a synthetic base diet plus an oral dose of *Malva parviflora* L. extract (500 mg/kg body weight/rat/day) (Ibrahim et al., 2021).
- **Group (2):** STZ-induced diabetic rats fed a synthetic base diet plus an oral dose of *Malva parviflora L. nano*-formulation (500 mg/kg body weight/rat/day) (Ibrahim et al., 2021).

2.2.8.5. Blood sample collection. After completing the two-month study period, all animals were starved for 12 h before being anesthetized with ketamine hydrochloride (35 mg/kg, i. m) and then euthanized by cervical dislocation. A blood sample was obtained from each animal's tail to test biochemical parameters, and the blood sample collected had a concentration of around 5 mL. At 4000 rpm, separation of serum and plasma was done for 15 min (Sigma Labor Centrifuge GMBH, West Germany, model 2–153360 osterode/Hertz) and stored at -20 °C.

2.2.8.6. Biochemical parameters. Insulin, Tumor necrosis factor (TNF-α), and Interleukin-6 (IL-6) ELISA kits were obtained from Abcam, UK. Glucose was evaluated by the enzymatic colorimetric method (Trinder, 1969). Hb was assessed by the enzymatic colorimetric method (Drabkin and Austin, 1932). The enzymatic colorimetric technique has been used to assess total cholesterol, HDL, LDL, and Triglycerides as lipid profiles (Richmond, 1973; Allain et al., 1974; Lopes-Virella et al., 1977; Wieland and Seidel, 1983). Colorimetric assays have been used to evaluate the activities of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase ALP as indices of liver function (Reitman and Frankel, 1957; Babson et al., 1966). As indications of liver function, colorimetric assays were used to evaluate plasma albumin (A), total protein, and total protein (Rheinhold & Seligron, 1953; Doumas et al., 1971). As indications of renal function, colorimetric techniques analyzed the levels of creatinine, urea, and uric acid (Fawcett and Scott, 1960; Schirmeister, 1964; Barham and Trinder, 1972). Superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Aebi, 1984), glutathione peroxidase (GPx) (Paglia and Valentine, 1967), and reduced glutathione (GSH) (Aykaç et al., 1985) were identified based on colorimetric techniques.

2.2.9. Histopathological examination

Specimens of the liver, pancreas, and spleen from all experimental rats were collected, fixed in neutral buffered formalin 10%, washed, dehydrated, cleared, and embedded in paraffin. At a thickness of 5 microns, sections of the paraffin-embedded blocks were prepared and stained with Haematoxylin and Eosin for light microscopic examination (Olympus BX50, Tokyo, Japan) (Bancroft and Gamble, 2008). Some modifications scored histopathological lesions semi-quantitatively (Mohammed et al., 2021). Briefly, lesions in ten fields were chosen randomly from each slide for each rat and averaged (score scale: 0 = normal; 1 = 25 %; 2 = 26–50 %; 3 = 51–75 %; and 4 = 76–100 %).

2.2.10. Statistical analysis

Each value is the mean±the standard error (n = 6). All biochemical parameters' variations were assessed statistically using one-way analyses of variance (ANOVA) and post hoc multiple comparisons with the Duncan test in the SPSS/PC software (version 22.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Evaluation of the nutraceutical potential

Plants are constantly being used to treat human ailments. Since the last century, scientific interest in phytotherapy has grown in several medical fields such as oncology, hematology, and immunology; the use of plants in medicine has influenced the identification of natural compounds (Balunas and Kinghorn, 2005).

One of the most significant medicinal plants in the world, *Malva parviflora* L. due to its abundance of bioactive molecules and critical biological properties.

The antioxidant effect of alcoholic extracts from plant leaves is associated with the availability of antioxidants like carotenoids, phenolics, and flavonoids. Specifically, Table 1 in this study illustrates the phenol and flavonoid content of 10.98 ± 0.52 GAE/g extract and 5.64 ± 0.84 mg catechin/g extract, respectively. *Malva parviflora* L. leaves contain high concentrations of flavonoids, which are associated with

Table 1. Total phenolic compounds, flavonoids and activity of *Malva parviflora* L. leaves.

Phenolic, flavonoid content and antioxidant activity	*Malva parviflora* L. leaves
Total Phenolic Content (mg GAE/g extract)	10.98 ± 0.52
Total Flavonoids content (mg catechin/g extract)	5.64 ± 0.84
DPPH (IC_{50} μg/ml)	120.7 ± 0.26

**Values are the mean of experiments performed in triplicate and are presented as mean ± SD.

Table 2. Phenolic compounds profile of the three alcoholic extracts of *Malva parviflora* L. by HPLC.

Compounds	Conc. (μg/g extract)	Compounds	Conc. (μg/g extract)
Gallic acid	214532.39	**Ellagic acid**	21146.89
Chlorogenic acid	96283.15	**Coumaric acid**	0.00
Catechin	26007.54	**Vanillin**	10931.36
Methyl gallate	6287.19	**Ferulic acid**	6120.48
Coffeic acid	11326.45	**Naringenin**	47950.86
Syringic acid	14673.83	**Taxifolin**	10425.44
Pyro catechol	58936.20	**Cinnamic acid**	59222.46
Rutin	105505.31	**Kaempferol**	666.48

solid antioxidant potential in scavenging free radicals (IC50: 120.7 ± 0.26 g/ml) (Table 1). A diet high in vegetables and, eventually, antioxidant components are widely established to lessen the incidence of numerous illnesses such as cancer and coronary artery disease (Duthie et al., 2000). Identification of the substances that promotes a healthy diet is thus crucial.

There was a significant variation in the previous report on the total phenol and flavonoid content in the ethanol extract of the *Malva parviflora*, reported to be 2.44 mg GAE/g and 1.07 mg RHE/g, respectively (Olajide et al., 2012; Sanghai et al., 2013), yet in another report by Farhan et al. (2012) the total phenolic content of *Malva* leaves varied from 1.99 ± 0.070. Moreover, 2.24 ± 0.031 according to the solvent, while the total flavonoid content ranged between 0.83 ± 0.063 and 1.07 ± 0.031. Flavonoids are a group of chemicals present in the extracts from plant origin and characterized by possessing powerful antioxidant properties. Antioxidants have the power to combat free radicals and inhibit cell deterioration brought on by free radicals (Dixit et al., 2007). A previous report by Farhan et al. (2012) revealed that *M. parviflora* L. leaves extract had an 88% scavenger activity rate and showed high antioxidant activity as ascorbic acid. While in our study, the DPPH scavenging activity was 120.7 ± 0.26 (IC50 μg/ml).

Chromatographic fingerprinting is a thorough and measurable identification approach that attempts chemical plus analytical methods to disclose the chemical composition of herbal ingredients via spectrograms and chromatograms (Ma et al., 2011). For the fingerprint analysis, the HPLC fingerprint approach was utilized to efficiently detect the phenolic components of *Malva parviflora* L. leaves extract.

As far as we know, there are not any researches to identify the phenols in *Malva parviflora* L. Using HPLC, the alcoholic extract of *Malva parviflora* L. leaves was able to reveal a total of sixteen unique components (Table 2). Among these 16 peaks, seven peaks have relatively high intensity, identified as Gallic acid, Rutin, chlorogenic acid, cinnamic acid, pyrocatechol, naringenin, catechin (214532.39, 105505.31, 96283.15, 59222.46, 58936.20, 47950.86, 26007.54 μg/g extract, respectively) by comparing each peak's UV spectrum and retention time to reference components (Table 2).

A literature study showed that most flavonoids have extreme antioxidant activity. Indeed, IC50 values concerning the radical scavenging activity of DPPH of isorhamnetin-7-O-glucoside, isoramnetin, quercetin-3-O-glucoside, and quercetin-3-O-rutinoside were 0.83, 5.54, 5.22, and 1.7 mg/ml, respectively. Therefore, consumption of *Malva parviflora* L. in the diet provides antioxidant activity concerning health.

Malva parviflora L. is an important medicinal plant due to its abundant bioactive ingredients and critical biological properties. *Malva parviflora* L. can be used as a supplement or additive to increase the nutritional potential in the context of functional foods' phenolic content and antioxidant potential.

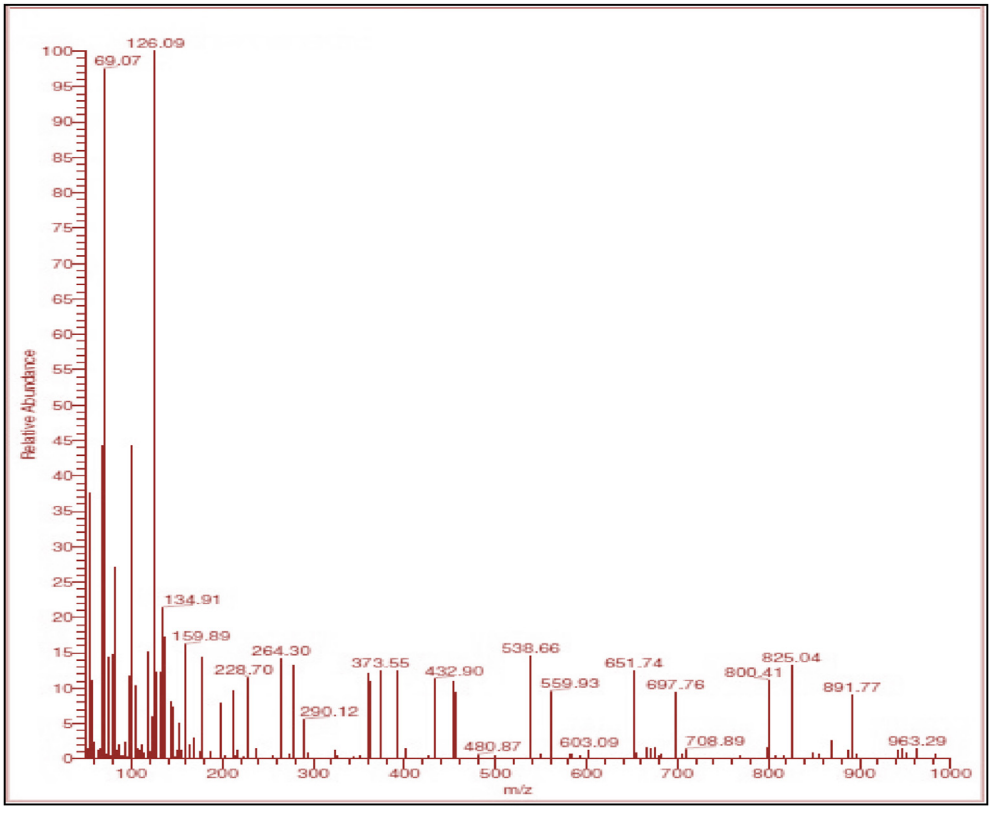

Figure 1. The mass spectrum of *Malva parviflora* L. leaves extract nanoparticles. Where, Ion Mode: = EL+, RT: 2.11 min, AV: 1, NL: 2.76E3, and Full m/z range: 50.00–1000.00.

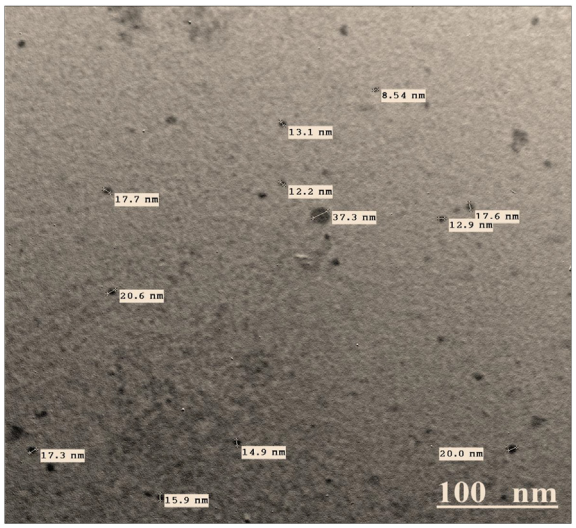

Figure 2. TEM micrographs of *Malva parviflora* L. leaves extract nanoparticles.

3.2. Nano-formulation characterization

The shape and size of the nanoparticles were assessed via mass spectrometry and transmission electron microscopy, and the zeta potential was evaluated using a Refractive Index Zeta Sizer.

3.2.1. Mass spectrometry (MS) analysis

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3.2.2. Transmission electron microscopy (TEM) analysis

TEM was used to depict the form and size of the resultant particles; Fig. (2). The TEM micrographs confirmed the existence of *Malva parviflora* L. extract with diverse particle diameters below 100 nm. The particle sizes were estimated to be about 22–54 nm based on TEM micrographs. The particles were round and spherical.

3.2.3. Refractive index Zeta Sizer

Nanomaterials have a greater surface area than their bulk counterparts with nanoscale effects, making them viable plant improvement tools. Owing to their peculiar size, shape, chemical composition, surface structure, charge, solubility, and agglomeration, nanomaterials differ from their bigger counterparts in terms of physicochemical and biological characteristics, which may have a significant impact on how they interact with biomolecules and cells. With nano-medicine, early detection and prevention, better diagnosis, correct treatment, and follow-up of illnesses are achievable. This study uses *Malva parviflora* L. as nanoparticles with enhanced effectiveness to treat an ulcer in a rat model study. The nanoparticles' large surface area to volume ratio is unique when the nanoparticle size ranges from 10 to 100 nm.

In Figures 1, 2, and 3, Mass spectrometry, transmission electron microscope, and zeta Sizer techniques were used to investigate the properties and morphology of *Malva parviflora* L. extract. We confirmed the existence of *Malva parviflora* L. extract with diverse particle diameters below 100 nm. The particle sizes were estimated to be about 7–20 nm with a round and spherical shape and no change in chemical composition (Figure 1 and 2). The Zeta Sizer Nanoparticle characterization system measures particle size, ζ-potential, and molecular weight. Depending on Zeta Sizer results for *Malva parviflora* L., which were Z-average (d.nm) = 50.3, Pdl = 0.48, particle size (d.nm) = 50.7 with a % Number = 99.8% and ζ-potential = -76.9, we confirmed that *Malva parviflora* L. are monodisperse nanoparticles with a nanoscale of less than 100 nm observed (Figure 3). The magnitude of the ζ-potential of a colloidal system is linked to its physical stability; if particles in a solution have a large ζ-potential (negative or positive), they repulse each other and reduce the probability of forming aggregations. Physical constants, such as the SL state, are considered to exist for particles having a ζ-potential larger than 30 mV (positive or negative). In contrast, ζ-potential close to 20 mV (positive or negative) indicates low colloidal suspension steady, and rapid particle aggregation may be included in the range of 5 mV to +5 mV (Mishra et al., 2009).

3.3. Antimicrobial activity

The results in Figures 4 and 5 represent the growth inhibition of the tested fungal strains by different concentrations of *Malava parviflora* L. extract and nano-formulation.

The antifungal activities of different concentrations of *Malva parviflora* L extract and *Malva parviflora L nano*-formulation were tested against pathogenic fungi. The obtained results showed that *Fusarium culmorum* was the most sensitive fungi to all the tested concentrations, either in the extract or in the nano-formulation, as the growth inhibition varied from 14.8 ± 0.78 to 21.2 ± 0.55 at concentrations of 100 µl–800 µl of the extract, respectively, while the use of the nano-formulation showed higher inhibition of fungal growth, ranging between 19.6 ± 0.35 to 28.2 ± 0.44 at concentrations of 100 µl and 800 µl respectively. *Aspergillus flavus* was the second most sensitive of the tested fungi, giving growth inhibition that varied between 13.2 ± 0.45 and 16.8 ± 0.8 of *Malva parviflora* L. extract at concentrations of 100 µl–800 µl, respectively. In comparison, it showed higher sensitivity to the nano-formulation as the

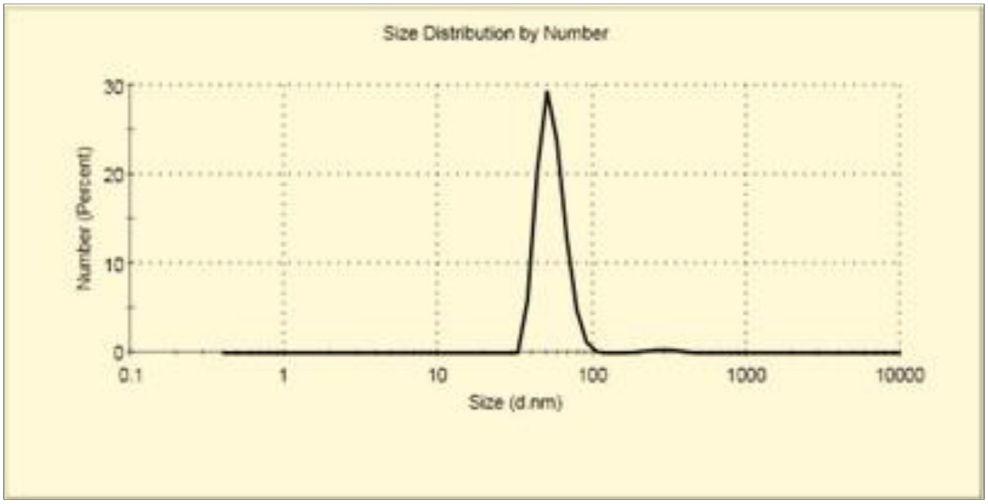

Figure 3. *Malva parviflora* L. leaves extract nanoparticles Zeta Sizer.

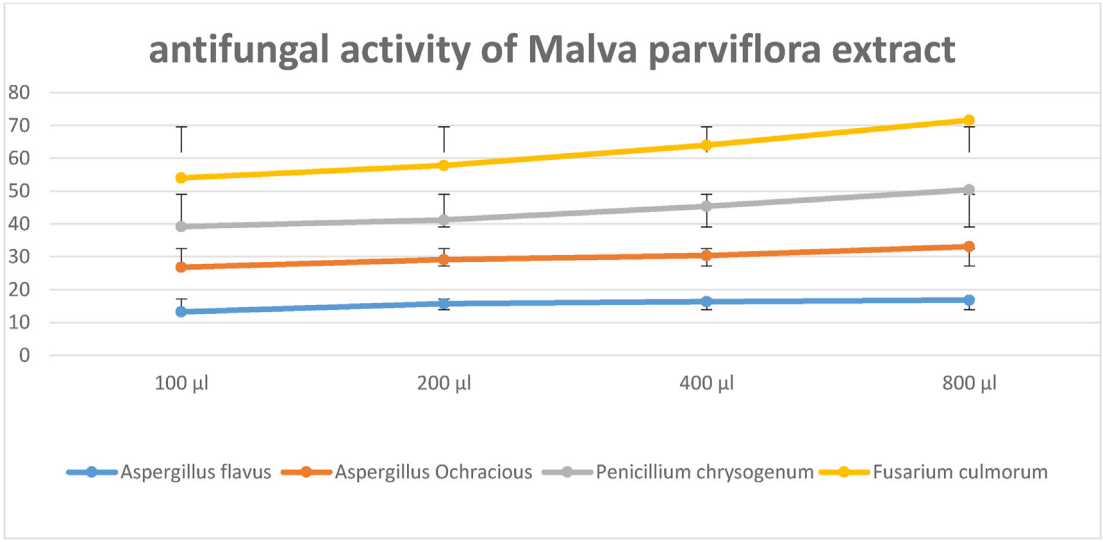

Figure 4. Antifungal activity of *Malava parviflora* L. extract.

inhibition was higher, giving 18.1 ± 0.4 to 20.6 ± 0.31 at concentrations of 100 µl–800 µl of the nano-formulation, followed by *Aspergillus Ochracious* which giving growth inhibition that varied between 13.5 ± 0.4 and 16.33 ± 0.75 of *Malva parviflora* L. extract, and that inhibition increased in the nano-formulation to 16.1 ± 0.06 and 23 ± 0.46 at concentrations of 100 µl–800 µl, respectively. The *Penicillium chrysogenum* inhibition ranged between 12.4 ± 0.06 and 16.6 ± 0.62 in the case of the extract, and that inhibition increased in the nano-formulation to 15.3 ± 0.12 and 21.1 ± 0.25 at concentrations of 100 µl–800 µl, respectively.

Regarding the antifungal activity of *Malva parviflora*, the results indicated high antifungal activity of either *Malva parviflora* extract or the nano-formulation against the tested fungal strains, which supports previous findings (Islam et al., 2010). The antimicrobial properties of *Malva parviflora extract are* attributed to the high phenolic content present in the extract. The outcomes of several earlier studies largely support the assertion that phenolic chemicals have a considerable influence on microbial development (Puupponen-Pimiä et al., 2001).

3.4. Nutritional parameters

The biological experiments were conducted to evaluate the therapeutic potential of *Malva parviflora* extract against type 2 diabetes mellitus. The results indicated a significant change in the nutritional parameters, although the food consumption between the tested groups was similar. Nevertheless, the final body weight and the weight gain were significantly lower in the STZ (diabetic) group, and that resulted from the diabetes case induced by STZ, which is known to cause a loss in body weight reduction resulting from structural protein degeneration, which also indicates a lack of food transformation into weight gain or growth attainment. The feed efficiency was more than that of the diabetic group, indicating better appetite and food conversion levels in the *Malva*-treated groups. Our results agree with the previous report (Eleazu et al., 2013).

The results in Table 3 showed the initial and final body weights and total food intake of the negative control, STZ-induced diabetic control (positive), and treated groups, which were checked up to an 8-week trial.

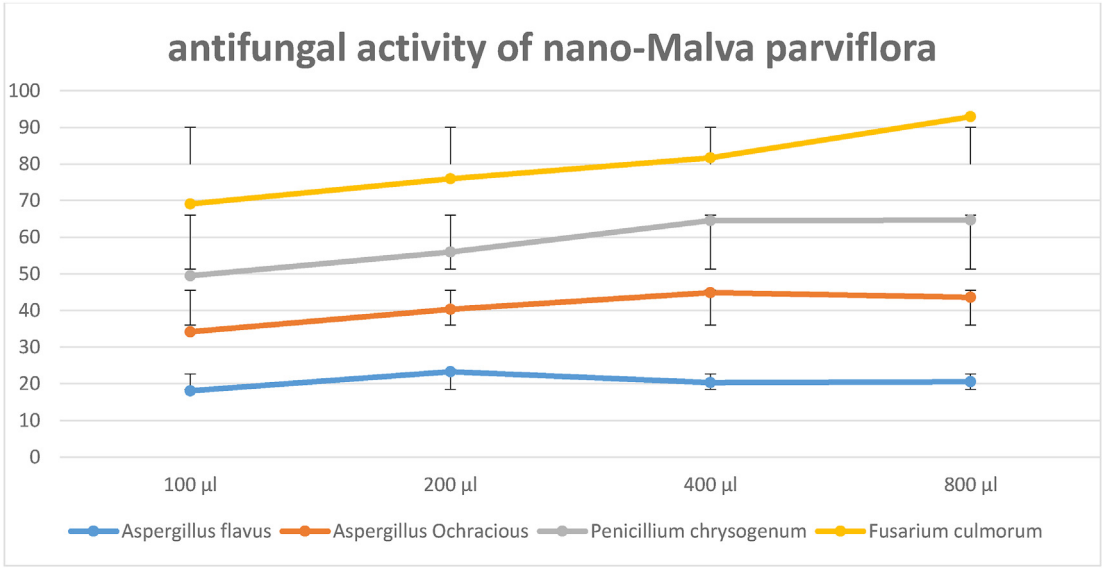

Figure 5. Antifungal activity of *Malava parviflora L. nano*-formulation.

Table 3. Effect of *Malva parviflora* L. extract and its nano-formulation on initial body weight, final body weight, and total food intake.

Group	Initial body weight (g)	Final body weight (g)	Body weight Gain (g)	Total food intake (g)	Food Efficiency
Negative control	216.2 ± 2.45^{a}	265.7 ± 3.58^{a}	49.5 ± 1.13^{a}	7135.5 ± 3.14^{a}	0.007 ± 0.36^{a}
STZ-induced diabetic control (positive)	215.9 ± 2.63^{a}	245.2 ± 3.77^{b}	29.3 ± 1.14^{b}	7100 ± 2.69^{b}	0.004 ± 0.42^{b}
Group (1)	215.5 ± 2.34^{a}	259.2 ± 3.45^{c}	43.7 ± 1.11^{c}	7140.2 ± 3.53^{c}	0.006 ± 0.31^{c}
Group (2)	215.3 ± 2.33^{a}	257.3 ± 3.57^{d}	42 ± 1.24^{d}	7123.8 ± 2.41^{d}	0.005 ± 0.51^{d}

Values are represented as Mean ± SE (n = 6) in which the same letters in each column reflect a non-significant difference across varieties, whereas different letters reflect a significant difference at $P \leq 0.05$. Food Efficiency= (Body Gain/ Total Food Intake).

No discernible differences existed in the initial body weight of rats in all the groups. At the end of the experiment, although the food consumption between all the tested groups was similar, the final body weight and weight gain were significantly lower in the STZ (diabetic) group than in the other treated groups. Also, the feed efficacy was significantly higher than in the diabetic group, indicating better appetite and food conversion levels in the *Malva*-treated groups.

3.5. Biochemical parameters

Administration of STZ leads to β-cell destruction, thus hindering insulin secretion begins within two days after administration, resulting in the diabetic stage (Eleazu et al., 2013). In Table 4, the plasma glucose and GHb results provided evidence of the successful experimental induction of diabetes mellitus. Development of the diabetic condition is related to the decrement of insulin secretion and the elevation of glucose levels in the blood, which is more verified by the elevation of GHb. A significant elevation in glucose and GHb was recorded, reaching 355.4 ± 2.74 and 13.6 ± 0.42, respectively, and a significant decrease in insulin levels to 9.82 ± 2.37. These are apparent markers for diabetes, yet all these parameters were reversed in the two groups receiving *Malva* extract. *Malva* extract decreased glucose level to 100.1 ± 3.39 and *Malva* extract nano-formulation decreased to 103.4 ± 2.47. At the same time, GHb levels were also reduced to 6.5 ± 0.48 and 7.9 ± 0.34 in the groups receiving *Malva* extract and *Malva* extract nano-formulation. Contrarily, though, a significant elevation in the insulin levels was recorded, reaching 15.23 ± 2.32 and 13.76 ± 2.34, respectively, which is a clear improvement. Glycosylated hemoglobin (GHb) concentrations evaluate the state of long-term glycemic control in people with diabetes over decades. Also, the dysfunction of β-cells is a fundamental reason for GHB elevation (Stakos et al., 2007). Our results stand in good harmony with previous reports (Grover et al., 2003; Perez et al., 2012). These parameters were restored to normal levels by restoring the liver's glucose-6-phosphatase, glucokinase, and hexokinase activities. Chronically elevated oxidative stress is a well-known consequence of diabetes mellitus (Banerjee and Saxena, 2012). TNF-α & IL-6 are groups of cytokines that control immunological and inflammatory responses and promote diabetes progression (Saxena et al., 2013). The immunological disorder is type 2 diabetes (T2D), in which cytokines play a significant role and cause defective insulin signaling and the selective death of beta-cells that produce insulin. A malfunctioning anti-inflammatory response may exacerbate T2D-causing chronic inflammation. This is a plausible explanation. Endocrinology and the modulation of inflammatory stress responses are crucial for the IL-6 family of cytokines (Spranger et al., 2003; Banerjee and Saxena, 2012; Wang et al., 2013; Lowe et al., 2014). Table 4 results indicated an extremely significant elevation in TNF

Table 4. Effect of *Malva parviflora* L. extract and its nano-formulation on Glucose, Insulin, GHb%, TNF-α, IL-6, Super Oxide Dismutase, Catalase, Reduced Glutathione and Glutathione Peroxidase.

Parameters	Group			
	Negative control	STZ-induced Diabetic control (positive)	Group (1)	Group (2)
Glucose (mg/dL)	98.8 ± 3.65^{a}	355.4 ± 2.74^{b}	100.1 ± 3.39^{c}	103.4 ± 2.47^{d}
Insulin (μU/ml)	17.23 ± 2.23^{a}	9.82 ± 2.37^{b}	15.23 ± 2.32^{c}	13.76 ± 2.34^{d}
GHb %	5.4 ± 0.65^{a}	13.6 ± 0.42^{b}	6.5 ± 0.48^{c}	7.9 ± 0.34^{d}
TNF-α (pg/ml)	97.35 ± 5.43^{a}	219.4 ± 3.31^{b}	112.2 ± 2.11^{c}	115.1 ± 2.32^{d}
IL-6 (pg/ml)	49.2 ± 2.69^{a}	185.1 ± 3.37^{b}	43.8 ± 3.48^{c}	42.1 ± 2.34^{d}
SuperOxide Dismutase (U/g)	3.2 ± 0.18^{a}	1.63 ± 0.09^{b}	2.87 ± 0.19^{c}	2.73 ± 0.24^{d}
Catalase (U/g)	163.2 ± 2.18^{a}	125.6 ± 3.26^{b}	156.7 ± 3.67^{c}	147.3 ± 3.95^{d}
Reduced Glutathione (U/g)	8.54 ± 0.28^{a}	2.11 ± 0.23^{b}	7.79 ± 0.21^{c}	7.23 ± 0.36^{d}
Glutathione Peroxidase (U/g)	9.34 ± 0.26^{a}	20.51 ± 0.99^{b}	11.69 ± 0.71^{c}	13.12 ± 0.63^{d}

Values are represented as Mean ± SE (n = 6) in which the same letters in each column reflect a non-significant difference across varieties, whereas different letters reflect a significant difference at $P \leq 0.05$.

in the STZ group, reaching 219.4 ± 3.31. This elevation was significantly lessened in the groups treated with *Malva* extract and *Malva* extract nano-formulation to 112.2 ± 2.11 and 115.1 ± 2.32, respectively, where the same trend was observed for IL6 as it was significantly increased in the diabetic group reaching 185.1 ± 3.37 and was reduced significantly after treatment with *Malva* extract and *Malva* extract nano-formulation to 43.8 ± 3.48 and 42.1 ± 2.34 respectively. Our results continued to support the previous report on the elevation of TNF and IL-6 in the STZ. This elevation was significantly lessened in the groups treated with *Malva* extract and nano-formulation.

Diabetes mellitus stimulates the liver to inevitably release more ROS due to processes brought on by hyperglycemia. Prior studies have revealed that diabetic animals' livers had dramatically enhanced oxidative damage to lipids and proteins (Feillet-Coudray et al., 1999; Mannan et al., 2014). According to several studies, free radical generation and antioxidant capacity are reduced in people with diabetes, which contributes to oxidative damage to cell components. Because of several causes, oxidative stress is exacerbated in diabetes. Among them, glucose autoxidation, which causes the generation of free radicals, is predominant (Rahimi et al., 2005). Such a case is present in our work as the results in Table 4 revealed a significant decrease in the STZ group in superoxide dismutase 1.63 ± 0.09, Catalase 125.6 ± 3.26, and reduced glutathione 2.11 ± 0.23. We were accompanied by a significant increase in glutathione peroxidase to 20.51 ± 0.99. These records were reversed after treatment with *Malva* extract and *Malva* extract nano-formulation for SOD significant elevation to 2.87 ± 0.19 and 2.73 ± 0.24, respectively. The same trend was observed for Catalase increased to 156.7 ± 3.67 and 147.3 ± 3.95 for both *Malva* extract and *Malva* extract nano-formulation, respectively, where the same was recorded for reduced glutathione. In contrast, Glutathione Peroxidase increased significantly in both *Malva* extract and *Malva* extract nano-formulation groups. Previous research documented that a decrement in SOD and CAT activities in a diabetic state increases ROS, leading to oxidation-inducing liver damage.

DM is usually related to a disturbance in lipid metabolism that takes place as a result of insulin deficiency or resistance. Insulin prevents fat metabolism by hindering intracellular lipase, which hydrolyzes tri-

Table 5. Effect of *Malva parviflora* L. extract and its nano-formulation on Total cholesterol, HDL, LDL, Triglycerides, Creatinine, Urea, AST, ALT, ALP, albumin, and total protein.

Parameters	Group			
	Negative control	STZ-induced Diabetic (positive)	Group (1)	Group (2)
Total cholesterol (mg/dL)	89.2 ± 3.48[a]	115.6 ± 3.56[b]	87.7 ± 3.27[c]	88.6 ± 5.16[d]
HDL (mg/dL)	47.4 ± 3.56[a]	32.6 ± 3.6[b]	46.1 ± 4.27[c]	46.9 ± 0.93[d]
LDL (mg/dL)	30.9 ± 2.61[a]	62.8 ± 3.24[b]	30.6 ± 2.82[c]	30.2 ± 3.64[d]
Triglycerides (mg/dL)	49.2 ± 2.38[a]	66.5 ± 3.53[b]	43.7 ± 3.42[c]	42.1 ± 3.65[d]
Creatinine (mg/dL)	0.91 ± 0.04[a]	0.96 ± 0.05[b]	0.86 ± 0.02[c]	0.89 ± 0.02[d]
Urea (mg/dL)	34.9 ± 1.36[a]	39.4 ± 1.21[b]	35.2 ± 1.14[c]	35 ± 1.27[d]
AST (U/L)	133.2 ± 1.46[a]	242.3 ± 1.24[b]	129.7 ± 1.23[c]	132 ± 1.29[d]
ALT (U/L)	93.9 ± 1.12[a]	205.4 ± 2.62[b]	113.4 ± 2.15[c]	105.6 ± 1.43[d]
ALP (U/L)	66.8 ± 3.24[a]	116.2 ± 3.32[b]	69.9 ± 2.46[c]	67.5 ± 2.11[d]
Albumin (g/L)	43.8 ± 1.35[a]	33.5 ± 1.27[b]	40.6 ± 2.72[c]	41.63 ± 1.27[d]
Total Protein (μmol/L)	12.34 ± 0.57[a]	6.76 ± 0.46[b]	11.63 ± 0.36[c]	12.07 ± 0.24[d]

Values are represented as Mean ± SE (n = 6) in which the same letters in each column reflect a non-significant difference across varieties, whereas different letters reflect a significant difference at $P \leq 0.05$.

glycerides to fatty acids, leading to increased cholesterol and triglycerides in the blood (Sah et al., 2011). In addition, hypercholesterolemia results from insulin depletion due to metabolic abnormalities (Sud et al., 2017).

Data in Table 5 reveals significant alteration of the tested biochemical parameters manifested in the lipid profile as there was a significant elevation in the total cholesterol in the STZ (diabetic) group, reaching 115.6 ± 3.56, where this elevation was attenuated significantly in the groups treated with either *Malva parviflora* L extract or *Malva parviflora L nano*-formulation, reaching 87.7 ± 3.27 and 88.6 ± 5.16 respectively. The same trend was observed in all the tested biochemical parameters except in the HDL, where it was reversed as the HDL decreased in the STZ (diabetic) group to 32.6 ± 3.6. In contrast, treatment with *Malva parviflora* L extract and nano-formulation led to the elevation of HDL to 46.1 ± 4.27 and 46.9 ± 0.93, respectively. LDL increased significantly in the STZ (diabetic) group to 62.8 ± 3.24, and treatment with either *Malva parviflora* L. extract, or its nano-formulation led to its reduction to 30.6 ± 2.82 and 30.2 ± 3.64, respectively. Treatment with the extract showed diminished triglyceride and total cholesterol levels and an enhancement in HDL compared to disease control rats. Our results support previous studies, which revealed the role of Gallic acid in lowering cholesterol, triglycerides, and low-density lipoproteins and improving insulin release by regenerating β-cells (Latha et al., 2011). Furthermore, since it is present in high concentration in the total phenolics measured in *Malva parviflora*, we could easily suggest that it is one of the main reasons for the treatment of both hyperglycemia and associated hyperlipidemia.

The liver enzymes that are well-known markers for liver cell damage to be released in the serum recorded significant elevation for both ALT and AST as observed in the STZ (diabetic) group, reaching 205.4 ± 2.62 and 242.3 ± 1.24 respectively. This elevation was reduced significantly in the *Malva parviflora* L. extract-treated group to 113.4 ± 2.15 and 129.7 ± 1.23, respectively, and in the *Malva parviflora* L., nano-formulation treated group to 105.6 ± 1.43 and 105.6 ± 1.43 respectively. This might be a consequence of the extract's defensive properties through its antioxidant effect in reversing liver damage induced by diabetes (Sunmonu and Afolayan, 2013).

Diabetes is a significant cause of many complications, including diabetic nephropathy. Roughly one-third of people with insulin-dependent diabetes have this condition (Kedziora Kornatowska et al., 2000). Many researchers have suggested that ROS significantly contributes to the progression of diabetic nephropathy. Mesangial cells produce hydrogen peroxide in response to high glucose levels and lipid peroxidation of glomerular mesangial cells, which also provides a rationale for diabetic kidney damage caused by oxidative stress (Anjaneyulu and Chopra, 2004). The kidney function was impaired in the STZ (diabetic) group, and that was evident by the elevation of all the tested kidney

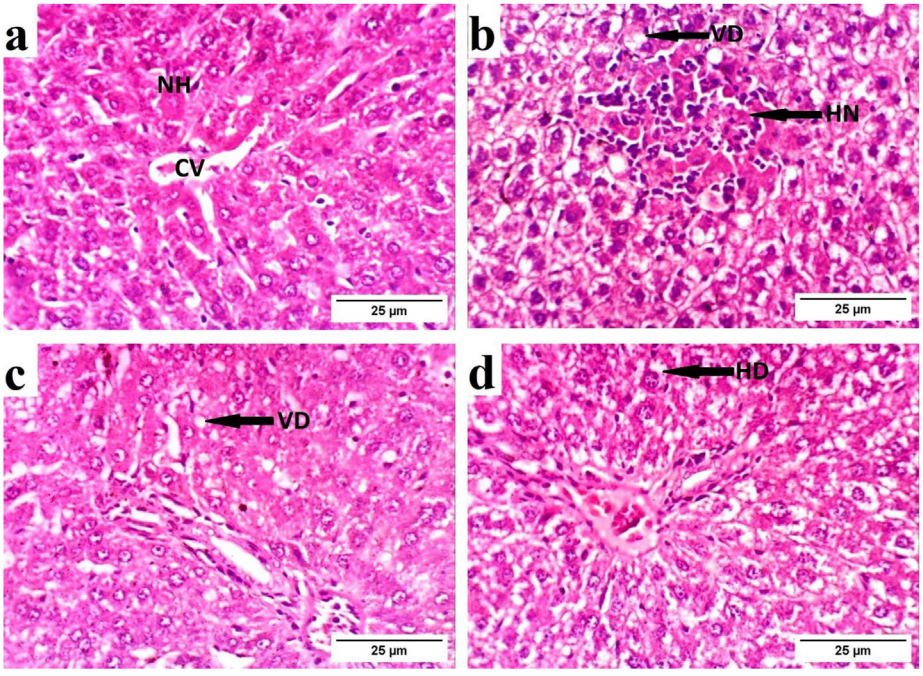

Figure 6. Representative photomicrographs of H&E-stained liver sections of rats: (a) negative control showing the normal histological architecture of hepatic parenchyma. (b) STZ-induced diabetic (positive), showing vacuolar degeneration of hepatocytes (VD) and focal hepatocellular necrosis associated with inflammatory cells infiltration (HN). (c) *Malva parviflora* extract, showing moderate vacuolar degeneration of hepatocytes (VD). (d) *Malva parviflora* nano-formulation, showing slight hydropic degeneration of hepatocytes (HD) **(scale bar, 25μm).**

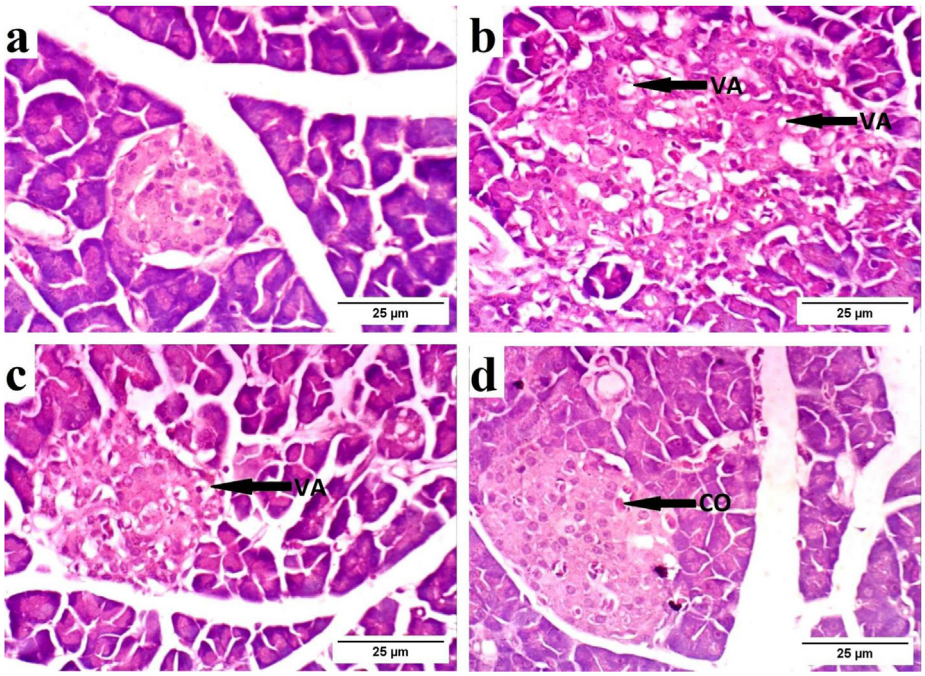

Figure 7. Representative photomicrographs of H&E-stained pancreas sections of rats: (a) Negative control showing the normal histological architecture of pancreatic tissue. (b) STZ-induced diabetic (positive), showing marked vacuolization of cells of islets of Langerhan's (VA). (c) *Malva parviflora* extract respectively, showing vacuolization of some cells of islets of Langerhan's (VA). (d) *Malva parviflora* nano-formulation, showing slight congestion of blood capillaries (CO) **(scale bar, 25 μm).**

function parameters, even though creatinine and urea were slightly elevated in the STZ treated group to 0.96 ± 0.05 and 39.4 ± 1.21, respectively, which was managed by the treatment with *Malva parviflora* L. extract to 0.86 ± 0.02 and 35.2 ± 1.14 and *Malva parviflora L. nano*-formulation 0.89 ± 0.02 and 35 ± 1.27 respectively yet the kidney deterioration expressed in the recorded decrease in albumin to 33.5 ± 1.27 and total protein 6.76 ± 0.46 and the increased ALP 116.2 ± 3.32 levels, while the records of the treated groups showed evident improvement in all kidney function parameters towards normal ranges and that mainly due to the high antioxidant activity of the extract where these outcomes align with prior findings (Ozkol et al., 2013).

3.6. Histopathological investigation

Microscopically, negative control rats' livers revealed the normal architecture of hepatic parenchyma, which comprises hepatocytes and

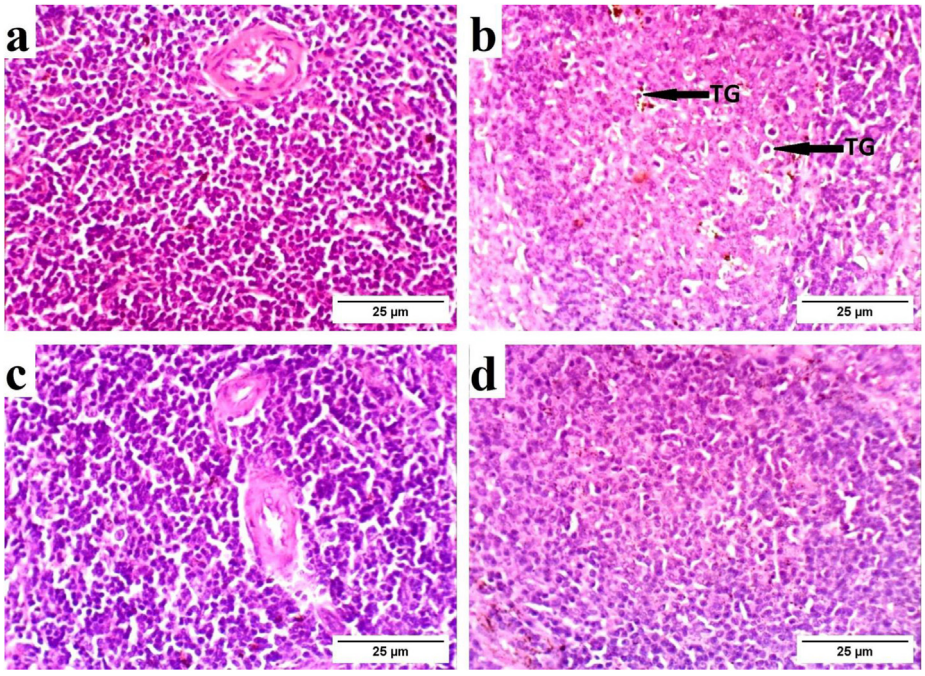

Figure 8. Representative photomicrographs of H&E-stained spleen sections of rats: (a) Negative control showing the normal histological structure of white pulp. (b) STZ-induced diabetic (positive), showing lymphocytic necrosis and depletion associated with presence of Tangible macrophages (TG). (c) *Malva parviflora* extract, showing no histopathological alterations. (d) *Malva parviflora* nano-formulation, showing normal lymphoid follicles **(scale bar, 25μm).**

Table 6. Histopathological lesions score in different experimental animals.

Organs	Lesions	Negative control	STZ-induced Diabetic (positive)	Group (1)	Group (2)
Liver	• Hepatocellular vacuolar degeneration	0	4	2	1
	• Hepatocellular necrosis	0	2	0	0
	• Inflammatory cells infiltration	0	2	0	0
Pancreas	• Congestion of blood capillaries	0	2	1	1
	• Vacuolization of the cells of islets of Langerhan's	0	4	2	0
	• Inflammatory cells infiltration	0	3	0	0
Spleen	• Lymphocytic necrosis and depletion	0	3	0	0
	• Presence of Tingible macrophages	0	2	0	0

central veins organized in hepatic cords (Figure 6a). On the contrary, the liver of diabetic rats showed various histopathological lesions described by Kupffer cell activation, hepatocellular vacuolar degeneration, and focal hepatocellular necrosis associated with inflammatory cell infiltration (Figure 6b). Meanwhile, an enhanced picture was noticed in the liver of rats treated with *Malva parviflora* extract; examined sections showed Kupffer cell activation and vacuolar degeneration of some hepatocytes (Figure 6c). Furthermore, marked regression of the lesions was noticed in the liver of rats treated with *Malva parviflora L. nano*-formulation revealed slight hydropic degeneration of hepatocytes (Figure 6d).

Concerning the pancreas, examined negative control sections revealed the normal histological architecture of pancreatic tissue (Figure 7a). Meanwhile, diabetic rats' pancreas revealed blood vessel congestion, marked vacuolization of cells of islets of Langerhan's (Figure 7b), and interracial inflammatory cell infiltration. However, the pancreas of rats treated with *Malva parviflora* extract showed more or less similar changes with diminished histopathological lesions; examined sections revealed vacuolization of some cells in the islets of Langerhans (Figure 7c). Furthermore, the pancreas of rats treated with nano-formulation exhibited no histopathological alterations except for slight congestion of blood capillaries (Figure 7d).

Microscopically spleen of negative control revealed the normal histological structure of white pulp (Figure 8a). In contrast, the spleen of diabetic rats revealed lymphocytic necrosis and depletion associated with the presence of Tangible body macrophages (Figure 8b). Meanwhile, the spleen of rats treated with either extract or nano-formulation exhibited restoration of the normal histology of the spleen with no histopathological alterations (Figure 8c & d). Table 6 summarizes the histopathological lesion scores in the rats' liver, pancreas, and spleen groups.

4. Conclusion

The obtained results in the current work gave precise data about the phenolic content of *Malva parviflora* and its nano-formulation and their potent antioxidant activity that offered significant benefits in diabetic control in the tested rats. Clinical studies are warranted to confirm the efficacy of *Malva parviflora* in the treatment of patients with diabetes. We also have to take into the consideration the oral bioavailability of *Malva parviflora* nanoformulation in human and the application of drug delivery system for the successful outcomes in clinical trials.

Declarations

Author contribution statement

Dina Mostafa Mohammed: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nesren Elsayed: Conceived and designed the experiments; Performed the experiments.

Doha H Abou Baker; Kawkab A. Ahmed; Bassem Sabry: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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