Phytomedicine 43 (2018) 126–134

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Original Article

The potential effect of garlic extract and curcumin nanoparticles against complication accompanied with experimentally induced diabetes in rats

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ARTICLE INFO

Keywords:
Diabetic cardiomyopathy
Aged garlic extract
Nano-curcumin suspension
Myocardial enzymes
Mn-SOD gene
RAGE gene
Rats

ABSTRACT

Background: Modified herbal medicines implicate the combination of several therapeutic practices of native systems of medicine that may extend many earlier generations, which frequently afford valuable therapeutic benefits.

Purpose: In this study, the role of nano-curcumin and aged garlic extract (AGE) as two modified phytomedicines on alleviating both of advanced glycation end products (AGEPs) and oxidative stress (OS) in streptozotocin (STZ) induced diabetic rats were investigated during this study.

Method: Nano-curcumin and AGE suspension were orally administrated at a dose of 300, 500 mg/kg body weight respectively. Serum glucose, insulin, total cholesterol, triglycerides and myocardial enzyme activities including creatine kinase-isoenzyme (CK-MB), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) were determined biochemically, while quantitative real-time polymerase chain reaction (qRT-PCR)-test had been used to determine relative of manganese-superoxide dismutase (Mn-SOD) and receptor for advanced glycation end products (RAGE) gene expressions in the heart tissue of rats. Structure of rat's heart tissue was examined by histopathological analysis (H&E).

Results: AGE increased the body weight and insulin concentration, while, it decreased serum glucose concentration, CK-MB, and LDH enzyme activities in comparing with the diabetic group. In addition, total cholesterol, triglycerides, and AST didn't show any significant changes in serum values of AGE compared to diabetic rats. Nano-curcumin suspension decreased the serum levels of triglycerides, CK- MB, LDH, and AST. While, there were non-significant changes in the body weight, glucose, insulin, and total cholesterol level of the same group compared with the STZ- untreated induced diabetic rats. The transcript quantity of manganese-superoxide dismutase gene (Mn-SOD) was highly accumulated (3.25 and 3.87-fold) in the heart tissue sample of the induced diabetic rats in response to both nano-Curcumin and AGE suspension respectively. While AGE was the most potent treatment where it caused down regulation of the receptor for advanced glycation end products gene (RAGE) expression (1.79-fold). Results of histopathological analyses under the light microscope showed restoring the structural integrity of the myocytes towards normalization in diabetic hearts treated with each of nano-curcumin and AGE suspension compared with the untreated diabetic heart samples.

Conclusion: Nano-curcumin and AGE suspension have a great therapeutic potential in the treatment of DCM, Diabetic cardiomyopathy, by attenuating cardiac inflammation, myocardial fibrosis, and programmed myocardial cell deaths through inhibiting OS and AGEPs accumulation in diabetic heart tissue. Furthermore, the hypoglycemic antioxidant properties of AGE resulted in more potent therapeutic effect than nano-curcumin in the treatment of diabetic hearts.

Abbreviations: AGE, aged garlic extract ; AGEPs, advanced glycation end products; OS, oxidative stress; ROS, reactive oxygen species; STZ, streptozotocin; CK-MB, creatine kinase-isoenzyme; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; qRT-PCR, quantitative real time polymerase chain reaction test; Mn-SOD, manganese-superoxide dismutase; RAGE, receptor for advanced glycation end products; DM, diabetes mellitus; CVDs, cardiovascular diseases; DCM, diabetic cardiomyopathy

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https://doi.org/10.1016/j.phymed.2018.04.039
Received 2 June 2017; Received in revised form 7 February 2018; Accepted 16 April 2018

Introduction

Herbal medicines involve the integration of several therapeutic experiences and practices of indigenous systems of medicine that may span many previous generations, which often provide valuable guidelines to the selection, preparation and application of herbal formulation with a view of providing therapeutic benefits (Chikezie et al., 2015).

Diabetes mellitus (DM) is a spectrum of metabolic disorders of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action, where it became a major health challenge worldwide (American Diabetes Association, 2011; Han et al., 2017). DM associated with hyperglycemia and oxidative stress which generally cause severe tissue damage and subsequently degenerative complications in many organs such as the heart (Jemai and Sayadi, 2015; Yan et al., 2017).

Cardiovascular diseases (CVDs) have been estimated as the leading causes for three quarters of deaths among diabetic patients (Westermann et al., 2009; Chi et al., 2012). Diabetic cardiomyopathy (DCM) is an independent diabetic cardiac complication, characterized by myocardial dysfunction (in the absence of coronary artery disease, hypertension, or valvular heart disease) including early-onset diastolic and late onset systolic dysfunction and it is associated with both type1 and type 2 diabetes mellitus (Huynh et al., 2010; Zeng et al., 2017). Hyperglycemia, lipid accumulation, excessive generation of reactive oxygen species, cardiac inflammation, apoptosis and cardiac fibrosis have been involved in the pathophysiology of DCM (Westermann et al., 2009; Falcao-Pires and Leite-Moreira, 2012; Das and Sil, 2013).

Recently, the effect of some natural products on the reduction of DCM through an antioxidative mechanism of action has been explored in previous studies (Parkash et al., 2014; Zhang et al., 2017; Yan et al., 2017). The hypoglycemic activity of a vast number of herbal plant products have been evaluated and confirmed in animal models (Chikezie et al., 2015). Herbal supplements with antidiabetic activity as garlic and curcumin might offer a natural keys to unlock diabetic complications (Laurance et al., 2006; Tachjian et al., 2010).

Aged garlic extract is an odorless alternative source of garlic, it has been shown to modulate cardiovascular risk factors in both clinical and preclinical settings (Steiner and Li, 2001; Morihara et al., 2011). Meanwhile, curcumin has been described as a treatment for diabetes and helped in decreasing the risk of CVDs in Ayurvedic and traditional Chinese medicine for thousands of years (Tachjian et al., 2010). Recently, nanoparticle technology has appeared as a prominent solution to curcumin poor bioavailability and its unwanted side effects (Ebtihal et al., 2014; Sankar et al., 2014; Ganugula et al., 2017). Superoxide Dismutase (SOD), the endogenous antioxidant enzyme systems, helps to manage the levels of Reactive Oxygen Species (ROS) in stressed cells (Houldsworth A., 2016). When the *RAGE* gene is up-regulated abnormally it plays crucial roles during the development of diabetes. *RAGE* gene is expressed at low levels in normal tissues, but becomes up-regulated at sites where its ligands accumulate (Xie et al., 2013).

Mainly, diabetic patients with poor glycemic control are at risk of various diseases, morbidity and mortality among people with diabetes mellitus are mostly triggered by cardiovascular disease (American Diabetes Association, 2010). Therefore, it is important to develop new therapeutics expected to decrease the development of DCM. The aim of this study was to determine the antioxidative effects of nano-curcumin, lipophilic polyphenolic, and AGE, hypoglycemic antioxidant, on the attenuation of DCM and regulation the mRNA relative quantity of *Mn-SOD* and *RAGE* genes as biomarkers for OS and AGEPs in chronic STZ- induced diabetic rats.

Materials and methods

Experimental animals

Seventy two adult white male albino rats (*Sprag dawly*) weighing between 220 to 240 g and 8 weeks of age were purchased from the animal house unit (Benha University, Faculty of Veterinary Medicine, Animal Breeding and Research Center). The rats were kept under a 12 h light-dark cycle and ambient temperature was maintained at 25 ˚C. Animals were allowed free access to water and were fed on uniformly basal diet. The animals were kept under hygienic conditions for at least two weeks for acclimatization before the beginning of the experiment.

Streptozotocin (STZ)

Streptozotocin powder purchased from Sigma-Aldrech with high purity (98%) Synonym: N-(Methylnitrosocarbamoyl)-α-D-glucosamine. STZ was used in this study to induce diabetic cardiomyopathy in rats with 60mg/kg body weight as a single dose. It was dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5) and injected within 5 min of preparation intrapretonialy (Ganda et al., 1976). It was obtained from MP Biomedicals, LLC, (Cat.No: 100557), USA.

Aged garlic extract (AGE kyolic) preparation and characterization

It was manufactured under license issued by the ministry of Health and Welfare of Japan, as the following steps: Sliced raw garlic was dipped into aqueous ethanol 15–20% and extracted for 2 months at room temperature in stainless steel tanks after separation of the solution the extract is generally concentrated and used (Amagase et al., 2001). Characterization of AGE based on the most common active ingredient (Allicin) by HPLC according to Bose et al. (2014) (Fig. 1). Chromatographic conditions consisted of Agilent HPLC 1200 serious, kromasil C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase was operated in isocratic mode (50:50 MeOH: H_2O) at a flow rate of 1 ml/min and wavelength 254 UV detector with ambient temperature 25˚ C for all operations. According to the present study Allicin concentration was 19.57 mg/ g AGE.

Curcumin nano-formulation

Curcumin

Curcumin 95% (total curcominoid content) from Turmeric Rhizome was obtained from Alfa Aesar, A Johnson Matthey Company (Catalog number. B21573, Lot. number.10186688, CAS number: 458-37-7).

Preparation of highly basic nanocurcumin

In Egyptian Petroleum Research Institute Cairo, Egypt, 1 M curcumin has low solubility in water was mixed with 4M sodium bicarbonate buffer, then grinded using mechanical ball mill (350 round/s) for 8 h. The color of curcumin changed from yellow to red as a result of the curcumin sodium salt formation. Curcumin nanoparticles were then dispersed into 50 ml of distilled water making aqueous solution which was filled in a reactor that was immersed in a water bath adjusted at 11 ˚C. Afterwards, this reactor was placed in an ultrasound apparatus (VCX-750 commercial sonicator) and sonication was applied in continuous mode at 100 Watt in a glass reaction vessel with thin and indented bottom for uniform and more efficient energy transmission (Hassan et al., 2013).

Characterization of nanocurcumin. Transmission electron microscopy (TEM) of the formed nano-curcumin sodium salt revealed average particle size of 200 nanometer (Fig. 2). Basicity showed high pH = 9.5 due to the formed nanocurcumin sodium salt and excess of sodium bicarbonate. As a result to this high basicity, nanocurcumin has been characterized by quick solubility in water and high penetration through

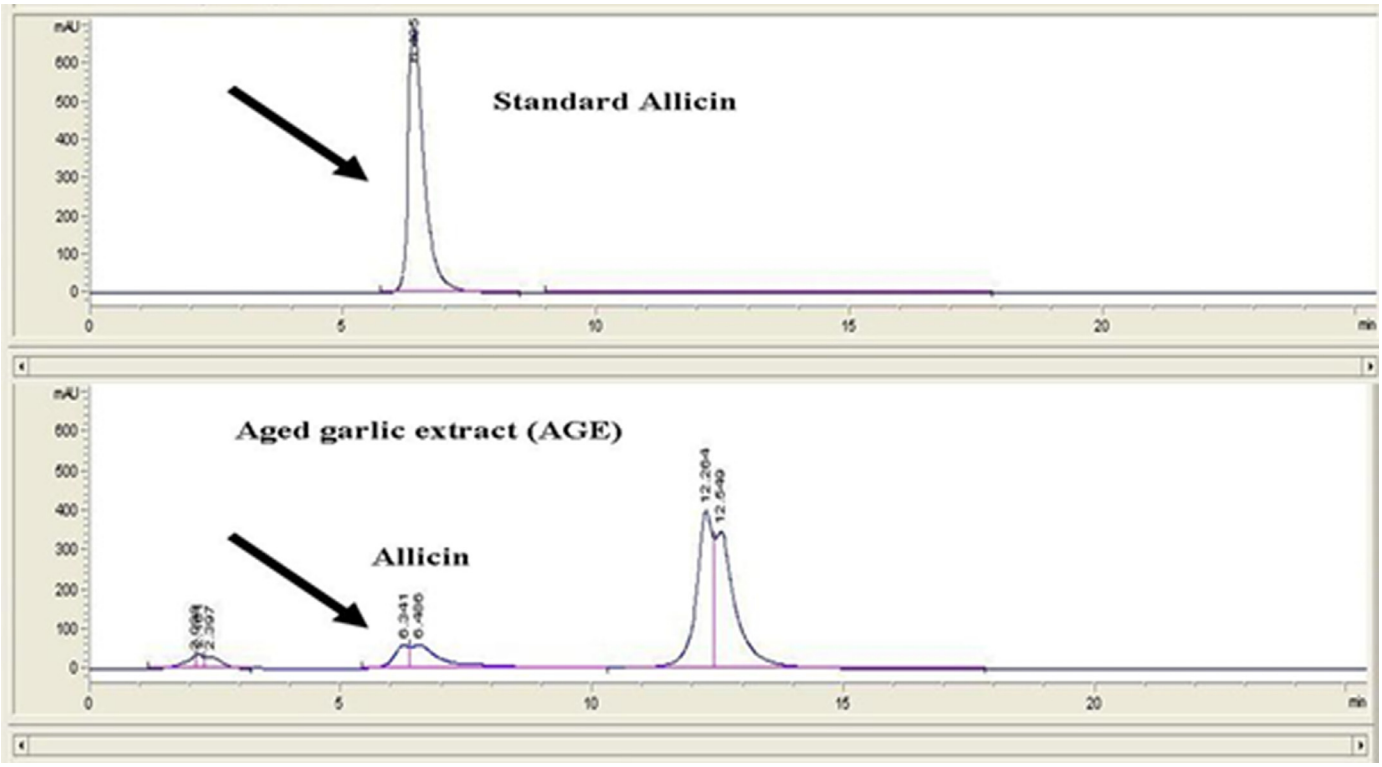

Fig. 1. Main active constituent (Allicin) of AGE separated by HPLC according to Bose et al. (2014).

the cell wall.

Bioavailability study

Twelve male albino rats weight from 200 ± 20 g; purchased from Faculty of Veterinary Medicine and allowed for one week adaptation before bioavailability study. Rats were divided into two groups 6 rats for each. The 1st was injected with curcumin 300 mg/kg b.w single dose (o.p). The 2nd group was injected with curcumin nano-form 300 mg/kg b.w. (o.p). The blood samples were collected at the different period as follow 0.5, 1, 1.5, 2, 4, 8, 12, 24h and curcumin was determined by HPLC according to Li et al. (2009). Tmax and Cmax of curcumin and nanoform were 120 min, 824 ± 23.74 μg/l plasma and 90 min, 1345.21 ± 37.62 μg/l respectively.

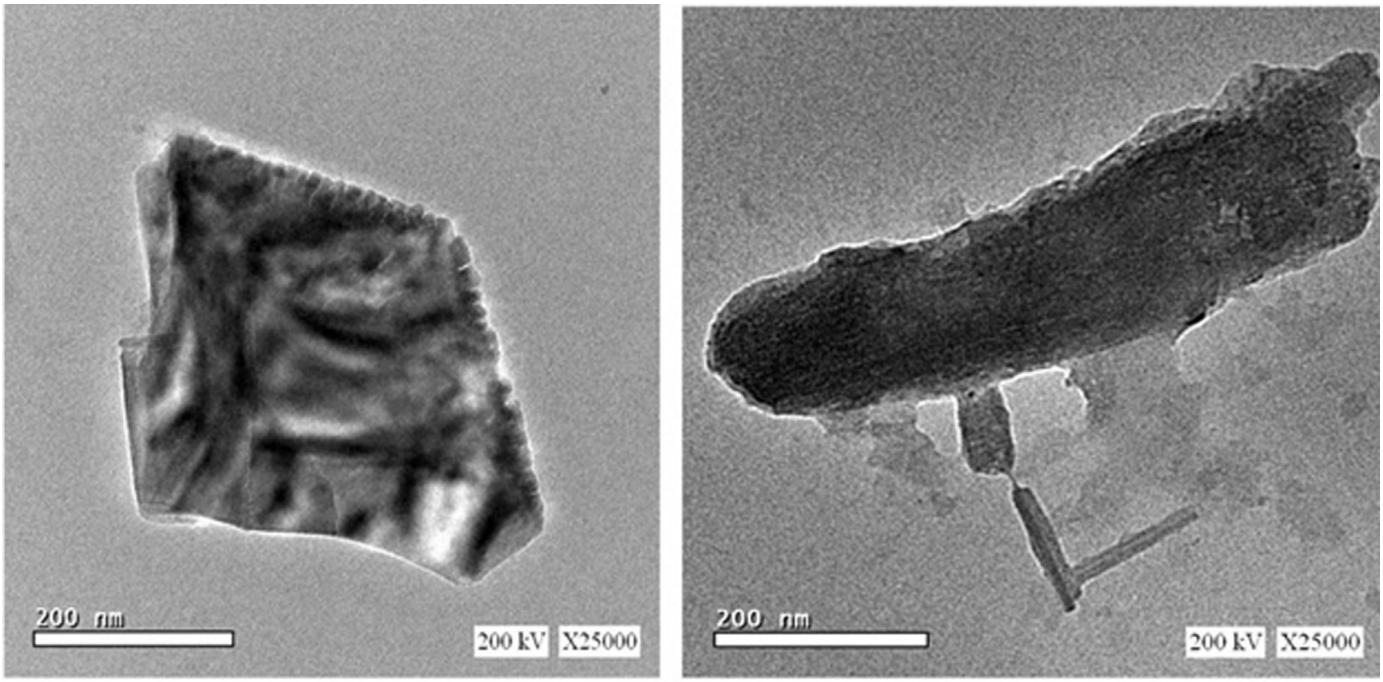

Fig. 2. Transmission electron microscope (TEM) image of curcumin nanoparticles showing particles size 200 nanometer.

Table 1
Oligonucleotide name and sequence of quantitative real time polymerase chain reaction (qRT-PCR).

Target gene		Sequence	Primer length
Mn-SOD	F	5′-GAA CCA CAG GCC TTA TTC CA-3′	20
	R	5′-GGG CTT CAC TTC ACT TCT TGC AAA C-3′	25
RAGE F R	F	5′-ATA GCC GCT CTG CTC ATT GG-3′	20
	R	5′-ATC ATG TGG GCT CTG GTT GG-3′	20
GAPDH F (reference gene) R	F	5′-TGT GAA CGG ATT TGG CCG TA-3′	20
	R	5′-TAA GCA GTT GGT GCA GG-3′	20

Induction of diabetes

Rats were subjected to hyperglycemia by intraperitonially (i.p) injection of freshly prepared STZ (dissolved in 0.1M citrate buffer, pH 4.5) as a single dose (60 mg/kg body weight) in a volume of 1ml/kg body weight. Three days after STZ injection; the blood samples were collected from retro-orbital venous plexus of eyes by using fine capillary glass tubes and used directly for blood glucose determination. Rats with blood glucose level ranged from 280–350 mg/dl were considered diabetic and included in the study (Ganda et al., 1976; Cam et al., 2003).

Experimental design

Rats were randomly divided into 4 equal groups (18 rats each) as the following:

Group 1: was injected with citrate buffer saline which was used in dissolving STZ and served as a control normal for all experimental groups.

Group 2: STZ-Diabetic rats were given saline by an oral gavage and served as a non-treated diabetic group.

Group 3: AGE- treated diabetic rats were administrated orally at a dose of (500 mg/kg b.w) (Shiju et al., 2013).

Group 4: Nano-curcumin treated diabetic rats were administrated orally at a dose of (300 mg/kg b.w) (Patumraj et al., 2006).

After fifty six days of treatments and in the basal fasting state, experimental rats of each group were weighed separately and average body weights were recorded.

Specimens collection

Random blood samples were collected from retro-orbital venous plexus of eyes in clean, dry screw capped tubes. Samples were allowed to coagulate at room temperature for 30 min and centrifuged at 3000 rpm for 15 min. The clean, clear serum was aspirated by pasture pipettes and received in dry sterile sample tubes, processed directly for glucose determination then kept in a deep freeze at -20°C until used for subsequent biochemical analysis.

Experimental rats of each group were anesthetized then sacrificed by cervical decapitation. Hearts were quickly removed; sections from the left ventricles were kept in ice cold saline, squeezing out the blood, plotted on filter paper, collected and stored at -80°C for gene expression. The remaining sections of the left ventricles were kept in10% neutral buffer formalin up to 15 days for histopathological examination.

Biochemical analysis

Serum glucose, insulin, total cholesterol, triglycerides, creatine kinase-MB, lactate dehydrogenase, aspartate amino transferase were determined according to the methods described by Trinder (1969); Baba et al. (1979); Allain et al. (1974); Schettler and Nussel, (1975); Urdal and Landaas (1979); Kornberg (1955); Reitman and Frankel (1957), respectively.

Total RNA extraction and complementary deoxyribonucleic acid (cDNA) synthesis

Heart Tissue samples from all studied treatments as well as control were grounded by Tissue Lyser LT apparatus (QIAGEN GmbH, QIAGEN Strasse 1, Hilden, Nordrhein-Westfalen-40724, and Germany) then total RNA was extracted from the suspension of cells using RNeasy® Mini kit (Qiagen cat.no.) following the manufacturer's protocol. Residual genomic DNA was eliminated by treating RNA with gDNA Wipeout Buffer which included in the QuantiTect® Reverse Transcription Kit, for effective elimination of genomic DNA contamination from starting RNA samples according to the manufacturer's recommendations. Reverse transcription (RT) of the RNA treated with gDNA was carried out using QuantiTect® Reverse Transcription Kit (Qiagen, Cat. No. 205311). Then total RNA and cDNA samples were stored at -80°C until use.

Differential expression analysis of Mn-SOD and RAGE genes by quantitative real time PCR (qRT-PCR)

This part of study aimed to quantify the relative amounts of *Mn-SOD* and *RAGE* genes transcripts in response to different treatments using specific primers designed for these two genes as well as the internal reference gene (Table 1). Triplicate PCR reactions were carried out for each analyzed sample in addition to non-template control (NTC) and cDNA template negative. Each PCR reaction consisted of, 2.5 µl of cDNA (except for NTC), 12.5 µl SYBR Green PCR Master Mix (QuantiTect SYBR Green PCR Kit, Qiagen Cat. no. 204143), 0.3 µM of each forward and reverse primer, 1µl RNase inhibitor and RNase-Free water to a final volume of 25 µl. Reactions were then analyzed on an Applied Biosystem 7500 Real time PCR Detection system under the following conditions: 95°C for 15 min and 40 cycles of 95°C for 30 s followed by 60°C for 1 min. The fluorescence monitoring occurred at the end of each cycle and finally 95°C for 15 min for melting temperature analysis. *GAPDH* gene was used as reference gene for qPCR data normalization. All experimentally induced changes in the studied genes expression are presented as n-fold changes relative to the corresponding controls. Relative gene expression ratios (RQ) between treated and control groups were calculated using the formula: $RQ = 2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001)

Statistical analysis

All data were presented as the mean ± Standard Error (SE). The data was evaluated by one-way ANOVA using SPSS (ver.16).The means were assessed for differences through least significant difference (LSD). The differences were considered statistically significant at $p < 0.05$. Percentage changes from control were calculated according to the calculation of [(Treatment – Control) / (Control)] X 100

Results

Results in Table 2 revealed the effects of aged garlic extract and nano-curcumin administrations on the body weight and serum values of STZ- diabetic rats as follows:

Body weight

STZ-induced diabetic rats showed a significant decrease in the body weight (214 ± 18.04) compared with the control normal rats. Administration of AGE showed a significant regain in the body weight (266 ± 12.93), while administration of nano-curcumin showed

Table 2
The effect of aged garlic extract (AGE) and nano-curcumin on the body weight and serum values of glucose, insulin, total cholesterol, triacylglycerol, creatine kinase-isoenzyme (CK-MB), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) in streptozotocin-induced diabetic rats.

Parameter	Group (1)	Group(2)	Group(3)	Group(4)
Body weight (gm)	402 ± 14.98[a]	214 ± 18.04[c]	266 ± 12.93[b]	242 ± 4.94[bc]
% changes from control	NA	−46.8 %	−33.8 %	−39.8 %
Glucose (mg/dl)	117.50 ± 4.40[b]	329.50 ± 42.86[a]	156.83 ± 28.06[b]	346.50 ± 11.04[a]
% changes from control	NA	181.6 %	34.0 %	195.7 %
insulin (µl/ml)	3.75 ± 0.11[a]	1.10 ± 0.18[c]	2.84 ± 0.40[b]	0.92 ± 0.66[c]
% changes from control	NA	−70.7 %	−24.3 %	−75.5 %
Total cholesterol (mg/dl)	98.50 ± 4.15	112 ± 17.18	87.67 ± 5.47	105 ± 5.04
% changes from control	NA	13.7 %	−11.1 %	6.6 %
Triglycerides (mg/dl)	115 ± 1.35[b]	144 ± 11.4[a]	143 ± 3.33[a]	124 ± 2.01[b]
% changes from control	NA	25.2 %	24.3 %	7.8 %
CK-MB (U/L)	129 ± 5.59[d]	604 ± 12.85[a]	482 ± 9.46 [b]	203 ± 5.04[c]
% changes from control	NA	368.2 %	273.6 %	57.4 %
LDH (U/L)	1118 ± 26.59[d]	4739 ± 120.17[a]	3045 ± 36.91[b]	1910 ± 7.97[c]
% changes from control	NA	323.9 %	172.4 %	70.8 %
AST (U/L)	87.50 ± 1.89[c]	174 ± 16.10[a]	149 ± 14[ab]	125 ± 12.13[b]
% changes from control	NA	98.9 %	70.3 %	42.9 %

Groups 1, 2, 3 and 4 are: Control normal, diabetic, aged garlic extract and curcumin-nano-suspension treated diabetic groups, respectively. Data are represented as (Means ± S.E).Values with different letters within the same row significantly differed at ($P < 0.05$). NA (Not applicable).

[a] Significant difference from Groups 1 at the same row.
[b] Significant difference from Groups 2 at the same row.
[c] Significant difference from Groups 3 at the same row.
[d] Significant difference from Groups 4 at the same row.

nonsignificant changes in the body weight (242 ± 4.94) compared with STZ-diabetic group.

Biochemical analysis

Glucose concentration

Data in Table 2 exhibited a significant elevation of glucose concentration (329.50 ± 42.86) in STZ-induced diabetic rats compared with the normal control. Administration of AGE showed a significant reduction in serum value of glucose (156.83 ± 28.06), while administration of nano-curcumin showed a nonsignificant change in glucose concentration (346.50 ± 11.04) compared to untreated diabetic rats.

Insulin concentration

A significant decrease in serum insulin concentration was determined in STZ-induced diabetic rats, (1.10 ± 0.18), compared with the normal group. Concerning to AGE-treated diabetic rats, the obtained results showed a significant elevation in serum insulin concentration (2.84 ± 0.40). However, there was a nonsignificant change in serum insulin concentration of nano-curcumin treated diabetic rats (0.92 ± 0.66) when compared to untreated group.

Lipids profile (total cholesterol and triglycerides)

Our findings revealed nonsignificant changes in serum total cholesterol level of all groups (STZ- diabetic rats, AGE, and nano-curcumin treated diabetic groups) compared with control normal one. On the other hand, STZ-induced diabetic rats showed a significant increase in triglycerides (144 ± 11.4) compared to the control normal group. Meanwhile, a nonsignificant effect on serum value of triglycerides was recorded in AGE-treated diabetic rats (143 ± 3.33) while there was a significant decrease of its value in diabetic rats treated with nano-curcumin (124 ± 2.01) compared to STZ-diabetic rats.

Myocardial enzymes (CK-MB, LDH, and AST)

Marked increase in CK-MB, LDH, and AST serum values (604 ± 12.85; 4739 ± 120.17 and 174 ± 16.10) was recorded in STZ-induced diabetic rats compared to the normal rats. Meanwhile, administration of AGE caused significant reduction in serum values of CK-MB and LDH with a nonsignificant effect on serum value of AST (482 ± 9.46; 3045 ± 36.91 and 149 ± 14) respectively. On the other hand, our findings revealed significant decrease in CK-MB, LDH and AST serum values in STZ- diabetic rats treated with nano-curcumin (203 ± 5.04; 1910 ± 7.97 and 125 ± 12.13) respectively, compared to untreated diabetic rats.

Fold changes of (Mn-SOD/GAPDH) and (RAGE/GAPDH) genes expressions in the left ventricle of rat's heart tissues

In this part of study, we investigated the antioxidant effects of AGE and nano-curcumin as two natural modified phytomedicines on the expression of *Mn-SOD* and *RAGE* genes in heart tissue samples of all the studied groups. *RAGE* gene expressed at low levels in normal tissues, while it up-regulated significantly ($P > 0.05$) at sites in which its ligands accumulate. The obtained findings in Fig. 3 indicated that *RAGE and Mn-SOD* genes were up-regulated (3.6 and 2.4-fold increase) respectively, in chronic STZ-diabetic hearts tissue compared with control normal hearts. While the mRNA quantity of *Mn-SOD* gene increased significantly ($P > 0.05$) (3.9 and 3.3-fold increase) in response to AGE and nano-curcumin administration respectively, to manage the levels of reactive oxygen species (ROS) in stressed cells. The positive up-regulation of *Mn-SOD* expression was accompanied with a significant ($P > 0.05$) decrease of *RAGE* mRNA values (1.79 and 2.34-fold increase) in AGE and nano-curcumin treated diabetic groups respectively compared to STZ-diabetic hearts. Results confirmed that AGE is more potent than nano-curcumin in regulating the oxidative markers in heart tissue samples.

Histopathological observations

Under the light microscope, massive necrosis of heart muscle fibers along with focal mass and fragmentation in addition to scattered nuclei were detected in cardiomyopathy diabetic hearts compared to control normal hearts. However, there was a general improvement in myocardial cell morphology with minimum pathological changes have been indicated in heart tissue sections of AGE and nano-curcumin treated STZ- induced diabetic rats compared to untreated diabetic hearts (Fig. 4. A, B, C and D) and (Table 3).

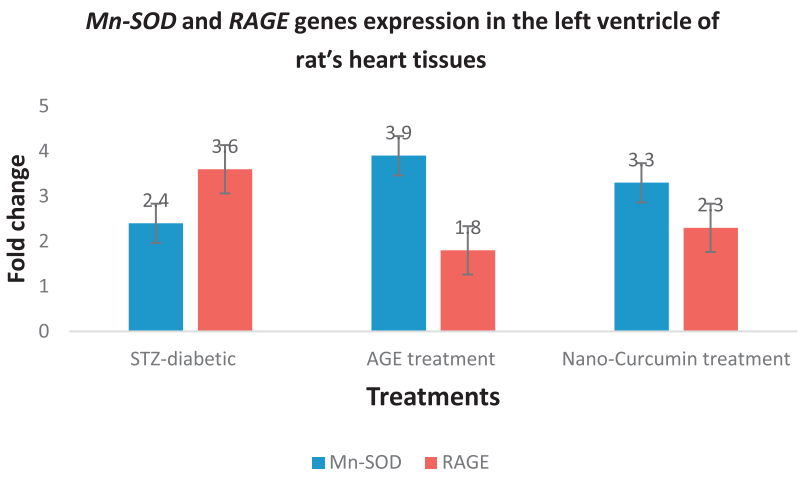

Fig. 3. Fold changes of *Mn-SOD* and *RAGE/GAPDH* genes expression in the left ventricle of rat's heart tissues, *GAPDH* gene was used as an internal reference gene.

Discussion

Diabetic cardiomyopathy (DCM) is a severe complication resulted from diabetes and cause high mortality (Yan et al., 2017).

In this study, the data recorded 3 days after intra-peritoneal (i.p) injection of STZ (60 mg/ kg b.w.) and at the end of the experiment (8 weeks). The course of the condition for (8 weeks) is necessary to study the mechanisms of the chronic changes in the cardiovascular system that resulted from STZ-induction in experimental rats (Wei et al., 2003). STZ causes a selective loss of the insulin secreting pancreatic β-cells via reactive oxygen species dependent oxidative damage which subsequent cause diabetes mellitus (DM) (Ghosh et al., 2015; Biswas et al., 2017). The toxic effect of STZ is due to its selective uptake into β cells via its low affinity glucose transporter (GLUT2) present in the plasma membrane, leading to inhibition of insulin secretion of beta cells, damaging the pancreas and glucose metabolism (Eleazu et al., 2013; Ghosh. et al., 2015)

STZ- induced diabetic rats showed a significant decrease in the body weight, these results are in harmony with findings of previous researchers who reported that rats lost weight and became over 50%

Table 3
Damage score of histopathological heart alteration for STZ-induced diabetic rat's model.

	Control	STZ-diabetic	AGE treated diabetic	Nanocurcumin treated diabetic
Necrosis	-	+ + +	+	+ +
Focal mass and fragmentation	-	+ +	-	+
Cell degenerations	-	+ +	+	+ +

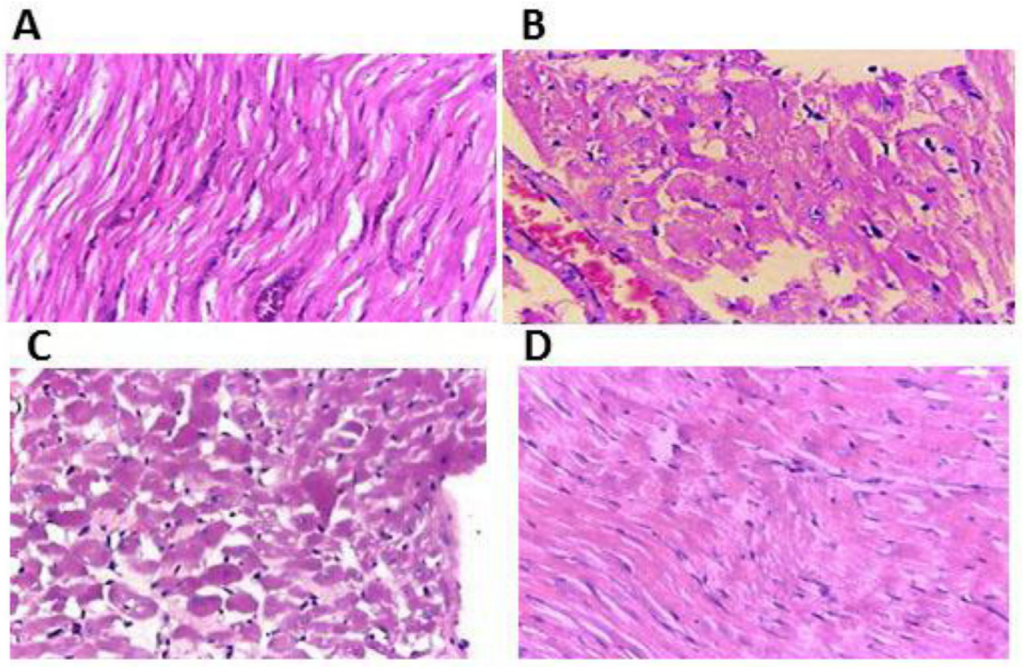

Fig. 4. Histopathological analyses of rat's ventricular heart tissue stained with Hematoxylin and Eosin (magnification = 4000).
A) Control normal group: normal architecture of heart, normal myocardial cell morphology with oval-elongated nuclei and no remarkable lesions.
B) STZ-diabetic group: massive necrosis of heart muscle fibers along with focal mass and fragmentation in addition to scattered nuclei
C) AGE treated diabetic group: minimum pathological changes include swelling of myocardial fibers and focal degeneration.
D) Nanocurcumin treated diabetic group: general improvements of myocardial cell morphology with less degenerations and cellular necrosis.

lighter after 8weeks of STZ-inducing diabetes, reduction in the body weight might be resulted from severe polyuria which accompanied the untreated hyperglycemia (Thomson et al., 2016, 2017). In addition to degradation of fat and structural proteins due to unavailability of carbohydrates for utilization as a source of energy (Juarez-Rojop et al., 2012).

Also, STZ-induced diabetic rats developed significant hyperglycemia and hypoinsulinemia. These results are in accordance with Thomson et al. (2016) who noticed continuous increase in the blood glucose level accompanied with approximately 11-fold decrease in serum insulin level in untreated STZ-diabetic rats compared to control normal. Furthermore, Eleazu et al. (2013) studied the chronic changes that accompanied STZ-induced diabetes to releasing of nitric oxide (NO), that mediates carbamoylation and alkylation of cellular components and destruction of pancreatic β islet cells through DNA damage and cell necrosis.

While there was a nonsignificant effect in total cholesterol level in STZ-induced diabetic rats, these findings are consistent with Wei et al. (2003); Yan et al. (2017) who reported that, STZ-diabetic rat mimics most of the chronic complications observed in the diabetic human; with explanation that glucose obtained from food cannot be utilized as an energy source by the body, so that the body uses energy from other sources, one of them lipid; insulin deficiency causes inhibition of lipogenesis and increased lipolysis resulting in mobilization of fatty acids from adipose tissue. Increased mobilization of fatty acids inhibits the glycolytic pathway, fatty acid synthesis, and encourages beta oxidation in liver to acetyl Co-A. High levels of acetyl Co-A in the liver would increase pathways that use it, the ketogenesis pathway and fatty acid synthesis.

Cardiac enzymes biomarkers (CK-MB; LDH, and AST) level increased significantly in STZ-untreated diabetic rats' serum. Results of the current study agreed with Suanarunsawat et al. (2016) who found that DM impaired the liver, kidney and cardiac functions of the diabetic rats by increasing serum levels of AST, ALT, creatinine, blood urea nitrogen, LDH, and CK-MB. Also, Badole et al. (2015) and Zeng et al. (2017) reported that, any serious insult to the heart muscle will enhance the release of AST, CK-MB, and LDH enzymes into the serum of diabetic animals. Likewise, Feng et al. (2008) and Zeng et al. (2017) suggested that peak rise in LDH and CK is proportional to the extent of injury to the myocardial tissue.

Also, the obtained results revealed up-regulation of *Mn-SOD* and *RAGE* genes expression in STZ-diabetic hearts compared to the normal heart tissue samples. During long standing hyperglycaemic state in diabetes mellitus, glucose interacts with the plasma proteins through a non-enzymatic process known as glycation forming what is called advanced glycation end products (AGEPs), adding that AGEPs play an important role in the pathogenesis of diabetic complications like cardiomyopathy, retinopathy, nephropathy, neuropathy (Parkash et al. 2014; Wautier et al., 2017). The obtained results came in a harmony with Yu et al. (2012); Yan et al. (2017) who stated that chronic hyperglycemia and DCM elevated markers of oxidative stress, malondialdehyde (MDA) and SOD. The significant increasing of mRNA values of *Mn-SOD* and *RAGE* genes in STZ-diabetic hearts have been attributed to chronic hyperglycemia which promote increasing of OS which has been suggested to include glucose auto-oxidation, formation of AGEPs along with the expression of RAGE, activation of protein kinase C (PKC) isoforms, increased activity of the hexosamine pathway and increased activity of the sorbitol pathway that in turn generates free radicals (FR) (Wautier et al., 2017). The interaction between RAGE and its ligands is thought to result in pro-inflammatory gene activation due to an enhanced level of RAGE ligands in diabetes, further during periods of elevated OS, cells protect themselves by increasing activity of various antioxidant enzymes. (Kain et al., 2010; Giacco and Brownlee, 2010; Yu et al., 2012; Badole et al., 2015; Biswas et al., 2017). SOD is the first line of defense that the cells use against OS and it is involved in the direct elimination of ROS by catalyzing the dismutation of superoxide radicals (Moussa, 2008).

Interestingly, our microscopic observations are in a accordance with Badole et al. (2015) and Ganugula et al. (2017) who detected marked degeneration of cardiac muscle and pancreas of STZ- diabetic rats. ROS usually damage different organs of the body by peroxidation of membrane lipids, oxidation of proteins, DNA and other intracellular macromolecules (Ganugula et al., 2017). Several mechanisms have been implicated in the myocardial fibrosis including RAGE activation results in inflammatory response, mainly by the activation of nuclear factor κB (NF κB), apoptosis, prothrombotic activity, expression of adhesion molecules, and oxidative stress (Ghosh et al., 2015).

Oral gavage of aged garlic extract (AGE) reduces the oxidative stress and other diabetes complications in STZ-induced rats (Thomson et al., 2017). Concerning the effect of AGE treatment on STZ-induced diabetic rats, our findings showed recovery in body weight after an initial loss following STZ injection. These results are in a harmony with Shiju et al. (2013); Thomson et al. (2016, 2017) who noticed that the STZ-diabetic rats treated with the 300,500 or 600 mg/kg b.w. doses of AGE stabilized their weight after an initial weight loss, with improving of polydipsia, polyphagia and polyuria. On the other hand, we found a significant reduction in the serum fasting glucose level accompanied with a significant elevation in insulin level in AGE-treated diabetic group. Our finding agreed with Thomson et al. (2016, 2017) and Ziamajidi et al. (2017) who reported that treating diabetic rats with both 600 mg and 2 g/kg b.w. AGE significantly decreased blood glucose and markedly increased serum insulin, due to the increasing in S allyl cysteine (SAC) and polyphenol compounds during aging of garlic which could be responsible for stronger antioxidant activity of AGE resulted in its hypoglycemic effect. While there were a non-significant decrease on each of total cholesterol and triacylglycerol levels. These results agreed with Borek, (2006) who detected that treatment of type 2 diabetic patients with 3000 mg of AGE daily had no effect on serum cholesterol and triglycerides after 3 months of treatment. Regarding to serum cardiac enzymes, oral administration of AGE to STZ- diabetic rats showed significant decrease in CK-MB and LDH while there was a non-significant decrease in AST. Where, the level of cardioprotection offered by the drug is associated with significant attenuation of plasma CK-MB and LDH levels (Majithiya and Balaraman, 2005). Borek, (2006) detected that the key ingredient responsible for the antiglycation properties of AGE was S-allyl cysteine (SAC), which proved an effective inhibitor of AGEPs, established antioxidant and free radical scavenger, in addition to increasing of antioxidant enzymes activity such as catalase, and glutathione peroxidase. Also, Capasso,(2013) and Thomson et al.(2016, 2017) found that increasing in SAC and polyphenol compounds during ageing cause increasing in AGE antioxidant properties involving the ability to scavenge reactive oxygen ROS and reactive nitrogen species RNS via increasing of enzymatic and non-enzymatic antioxidants levels, activating Nrf2 factor or inhibiting some prooxidant enzymes (xanthine oxidase, cyclooxygenase, and NADPH oxidase).

Oral administration of AGE to STZ- diabetic groups, reduced the dramatic cytotoxic effect exerted by STZ, where it was active in decreasing the hyperglycemia by-products AGEP and oxidative stress through significant up-regulation of SOD/GAPDH and significant down-regulation of RAGE/GAPDH genes expression. Thus our findings came in a harmony with Ziamajidi et al. (2017) who stated that AGE (2 g/kg b.w.) is a strong antioxidant where it contain compounds like S-allyl cysteine (SAC), therefore it down-regulate the expression of iNOS gene and NO level hence decreasing oxidative stress status. In addition, they found that the mRNA level of tumor necrosis factor alpha (TNF-a) was decreased in STZ diabetic rats in response to garlic treatment (2 g/kg/d, gavage) ($P < 0.01$), where it was close to the normal level.

Also, Thomson et al. (2016, 2017) reported that 100, 300 or 600 mg/kg as well as 2 g/kg b.w AGE positively reversed the diabetic changes in STZ-diabetic rats resulted in significant hypoglycemia and hyperinsulinemia with an increase of 9% in all the studied AGE doses. Also, our results are supported by the finding of Dillon et al. (2003);

Maldonado et al. (2003); Ahmad and Ahmed (2006); Ahmad et al. (2007) who reported AGE has an antioxidant potential in attenuating OS directly by reducing the formation of superoxide and lipid peroxidation, or by increasing of endogenous enzymes such as catalase and glutathione peroxidase. Similarly, Balamash et al. (2012) and Thomson et al. (2016) reported that treatment with either 300 or 600 mg/kg of AGE significantly increased the anti-oxidant defense systems by preventing formation of amadori products which can lead to protein oxidation, in addition to lowering of lipid peroxidation in diabetics. Similarly, Components of AGE (N-acetylcysteine) and AGE itself have been shown to reduce expression of RAGE, endothelial cell adhesion molecules, NF-κB, MDA, ROS generation and matrix metallo-proteinases in apoE-deficient mice (Lu et al., 2011).

Curcumin, natural and safe anti-inflammatory agent, reduces the complications associated with diabetes as well as cell death. However, very high doses need to be used for its poor oral bioavailability, which considered a critical barrier to translate the anti-inflammatory effects to clinical states. Therefore, preparing large scale of curcumin encapsulated nano-systems is a great demand (Ravichandran, 2013; Ganugula et al., 2017)

Concerning to the effect of nano-curcumin treatment in STZ-induced diabetic rats, the obtained results came in a harmony with Ganugula et al. (2017) who prepared biodegradable nano-systems encapsulating curcumin (nCUR), and tested the ability of nCUR to suppress STZ induced apoptosis and inflammation in rats' beta cells and pancreatic islets resulting in (9-fold) improvement in its oral bioavailability. Furthermore, Zhang et al. (2017) found that curcumin nanoparticle (Cur-NPs) markedly suppressed SAH-mediated oxidative stress and eventually reversed the induced cell apoptosis in rats. Where they studied the effect of curcumin (150, 300 mg/kg) as well as Cur-NPs (10, 20 mg/kg) administration and found that Cur-NPs (20 mg/kg) significantly increased the activities catalase, SOD, and GSH-Px, in rat SAH model which protect tissues from ROS damaging effects. The level of blood glucose at 72 h after STZ injection was significantly lower in nano-curcumin (nCUR) than curcumin groups may be due to the oral bioavailability improvement of 50 mg•kg-1 nCUR dose, reduction in glucose level was accompanied by an increase in insulin level, proposing enhanced pancreatic beta cell function in diabetic rats (Ganugula et al., 2017). The demand of using curcumin nano-particles is supported by the findings of Majithiya and Balaraman (2005) who found that curcumin has no effect on blood glucose, insulin and pressure in diabetic rats treated orally with (200 mg/kg b.w.) curcumin for 4 to 24 weeks in both of STZ and high fat diet (HFD)-induced diabetic rats. Concerning to lipid profile, our data agreed with Gutierres et al. (2012) detected that curcumin-supplemented yoghurt improves physiological and biochemical markers of STZ-diabetic models. Focusing on cardiac biomarkers (CK-MB; LDH and AST), our study agreed with Badole et al. (2015) who reported that the integrity of the cardiac apparatus in drug biotransformation and metabolism could be assessed by evaluating the levels of (AST, CK-MB, and LDH) in serum.

In the recent study, significant up-regulation of *Mn-SOD* accompanied with significant down-regulation of *RAGE* genes expression in nano-curcumin treated group was recorded. Few previous studies were conducted using nano-curcumin, in which the administration of 20 mg/kg nano-curcumin significantly increased the SOD and catalase activity in rat SAH model, furthermore, 50 mg/kg nano-curcumin suppressed apoptosis and inflammation in STZ-induced rats pancreatic beta cells (Zhang et al., 2017; Ganugula et al., 2017), respectively. Zhang et al. (2017) quantified the mRNA levels of different inflammatory agents using qRT-PCR in response to administration of 20 mg/kg nano-curcumin (Cur-NPs), resulted in the ability of Cur-NPs to block the SAH-elevated protein and reduce the mRNA levels of IL-1β, IL-6, and TNF-α in the brain of rat SAH model. Our results agreed with Hsuuw et al. (2005); Yu et al. (2012) who reported that curcumin attenuates OS, and normalizes enzymatic activities involved in lipid peroxidation. It was believed that the antioxidant effects produced by curcumin leading to prevent induction of ROS generation by inhibiting (Ca^{2+}) entry and (PKC) activity; thus, inhibit cellular apoptosis by blocking apoptotic changes, DNA fragmentation, caspase-3 activation, mitochondrial cytochrome C release, and JNK activation, an isoform of stress-activated protein kinase (SAPK) (Balasubramanyam et al., 2003; Chan et al., 2005; Hsuuw et al., 2005). While, Hsuuw et al. (2005); Hu et al. (2012) reported that Curcumin prevents AGEPs accumulation by trapping methylglyoxal and inhibiting (NFκB), thus reduces RAGE gene expression. Furthermore, curcumin restores trans-membrane potential and stiffened membrane fluidity causing limiting the release of pro-inflammatory factors in the presence of high glucose or increased concentrations of AGEPs, mainly at the late stages of diabetes (Margina et al., 2013).

Conclusion

The present study revealed that aged garlic extract (AGE) and nano-curcumin have great potential protective and therapeutic role in diabetic cardiomyopathy and other cardiovascular disorders. The two studied modified phytomedicines attenuated metabolic and myocardial enzymes disturbances, cardiac inflammation, fibrosis of myocardial and programmed cell deaths resulted in hyperglycemia. Furthermore, they have potent role in decreasing oxidative stress and hyperglycemia by products accumulation in diabetic hearts via regulating the expression of *Mn-SOD* and *RAGE* genes. In general, we noticed that the hypoglycemic antioxidant properties of AGE resulted in more potent therapeutic effect than the lipophilic polyphenolic effect of nano-curcumin in treatment of diabetic hearts.

Conflict of interest

There is no conflict of interest

Acknowledgment

Special thanks to Dr. Mercedes Bonfill, Prof. of Plant physiology/ Biotechnology, Faculty of Pharmacy, University of Barcelona (Spain) for her kind help and assistance in reviewing this manuscript. We would like to thank center of excellence in scientific research faculty of Veterinary Medicine, Benha university, especially the project that funded by Management Supporting Excellence and Benha university.

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