



Metformin Therapy Assisted by Nanotechnology as a Possible Combinational Treatment for Diabetes Mellitus-an Experimental Study

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Abstract

Background: Silver nanoparticles (AgNPs) have increased interest in various medical applications after it was found that they have a high capacity to inhibit many types of pathogenic bacteria and have anti-inflammatory and even anti-tumor activity. Also, gold nanoparticles (AuNPs) have been found to inhibit transforming growth factor- β and have anti-hyperglycemic/antiglycation, anti-inflammatory, antiangiogenic, and antioxidant effects.

Aims & Objectives: Investigating the effect of synergistic combination of nanotechnology together with metformin treatment in rats with high fat diet induced diabetes.

Methods: Sixty adult male albino rats, were obtained from the animal house in the Faculty of Medicine of Zagazig University. They were divided into groups. Control group (I): rats fed on normal diet. High fat diet induced diabetic obese group (II) for 3 months, was subdivided into, Untreated (IIa), Metformin treated (IIb), Combined metformin and gold nanoparticles treated (IIc), Combined metformin and silver nanoparticles treated (IId), Combined metformin, gold nanoparticles, and silver nanoparticles treated (IIe). Blood was collected for measurement of serum levels of glucose, insulin, HOMA IR (Insulin Resistance), HOMA β (β Cell Function), Serum total cholesterol, high-density lipoprotein- cholesterol, triglycerides, low-density lipoprotein-cholesterol and inflammatory markers.

Results: The mean values of insulin and glucose and HOMA IR were significant decreases in HFD/obese metformin, and gold nanoparticles treated ($P < 0.05$, $P < 0.05$, $P < 0.001$ respectively) and HFD/obese metformin, and silver nanoparticles treated groups ($P < 0.001$). In addition, there were significant increases in HOMA B in HFD/Metformin and gold nanoparticles ($P < 0.05$) in comparison to HFD/obese metformin treated groups. There were significant decreases in Serum total cholesterol, triglycerides, low-density lipoprotein-cholesterol and inflammatory markers in HFD/obese metformin, and gold nanoparticles treated.

Conclusion: combination of nanotechnology together with metformin improves glucose tolerance and dyslipidemia.

Keywords: metformin, nanoparticles, diabetes, dyslipidemia

Introduction

Diabetes mellitus (DM) is a rapidly growing health problem worldwide that is expected to impact 592 million people by 2035.¹ It is associated with an increased risk of a chronic inflammatory state and oxidative stress that affect many tissues.² The most common form of diabetes, type 2, is characterized by a systemic inflammatory disease accompanied by insulin resistance (IR) or decreased metabolic response to insulin in several tissues, including the adipose tissue, liver, and skeletal muscle, as well as by reduced insulin synthesis by pancreatic beta cells.³

Metformin

(<https://go.drugbank.com/drugs/DB00331>), a biguanide, is an oral anti-diabetic drug (first-line therapy) used to treat type 2 diabetes and has been shown to improve glycemic control, reduce hyperglycemia, and prevent the occurrence of diabetes-related complications.⁴ The mechanism of action of metformin may include the inhibition of glucose production in the liver, together with enhancing insulin sensitivity.⁵ New research has suggested that nanomedicine can improve the effectiveness of metformin therapy for type 2 diabetes.⁶

Nanomedicine is a branch of medicine that utilizes nanotechnology for the diagnosis, treatment, and prevention of diseases.⁷ The use of nanomedicine for drug delivery has shown promising results with lower dosages and increased efficacy of drugs.⁸ The unique properties of nanoparticles, such as their small size and high surface area-to-volume ratio, allow them to bind to specific targets in the body and release drugs more efficiently. This specificity can reduce drug toxicity and improve bioavailability.⁹

One possible way to use nanotechnology to improve metformin therapy is by using nanoparticles (NPs) to deliver the drug directly to the target tissues.¹⁰ Studies have shown that nanoparticles can effectively deliver

metformin to the liver, the primary target for treating diabetes.¹¹ In this way, the dose required to achieve therapeutic effect can be reduced, and the side effects can be minimized.¹² Silver nanoparticles (AgNPs) have increased interest in various medical applications after it was found that they have a high capacity to inhibit many types of pathogenic bacteria and have anti-inflammatory and even anti-tumor activity.¹³ Also, gold nanoparticles (AuNPs) have been found to inhibit transforming growth factor- β and have anti-hyperglycemic/antiglycation, anti-inflammatory, antiangiogenic, and antioxidant effects.¹⁴

The authors of this study were interested in evaluating the potential effect of the synergistic combination of nanotechnology (AgNPs/AuNPs) and the standard metformin treatment in albino rats with high-fat diet-induced diabetic obesity.

Material and Methods

Animals

Sixty adult male albino rats 12 weeks old, weighing 180–200 g, were obtained from the animal house in the Faculty of Medicine of Zagazig University. About six rats per cage were kept under hygienic conditions. Animals had free access to water; they were kept at room temperature and were maintained on a 12-light/dark cycle.¹⁵ The rats were accommodated in animal house conditions for one to two weeks before the experiments were conducted.

Protocol of the experiment and diabetes induction

The animals were divided into two main groups: Group I (control; $n = 10$): the rats were fed on a regular diet, which consisted of 25.8% protein, 62.8% carbohydrate, and 11.4% fat. Group II ($n=24$): the rats were fed on high-fat diet-induced obesity; (58% of diet calories

derived from fat, 18% from protein, and 24% from carbohydrates; 5.6 kcal/g) for 12 weeks. These animals were subdivided into four subgroups (each n = 10): (1) Group IIa: rats were fed HFD, (2) Group IIb: rats were fed HFD with metformin therapy 500 mg/kg, IP, daily for four weeks,¹⁶ (3) Group IIc: rats were fed HFD with Metformin therapy and gold nanoparticles by oral administration of 0.75 mg/kg gold nanoparticles synthesized using *C. cuspidatus* for 21 days,¹⁷ and (4) Group IId: rats were fed HFD with metformin therapy and silver nanoparticles by oral administration (10 mg/kg b.w daily) for 28 days.¹⁸ Then the rats were sacrificed by decapitation after 12 h of fasting under anesthesia [ketamine (80 mg/kg)].

Blood samples were obtained by exsanguination at the time of the sacrifice, collected, and allowed to clot for two hours at room temperature before centrifugation. Sera were stored at -20°C until analysis.

Laboratory analysis

For all animals, the following parameters were measured: fasting serum insulin level, fasting serum glucose level, Calculation of homeostasis model assessment (HOMA) and β -cell function (HOMA- β) [HOMA-IR = $\text{insulin } (\mu\text{U/mL}) \times \text{glucose (mmol/L)} / 22.5$] and β -cell function [HOMA- β = $20 \times \text{insulin } (\mu\text{ U/mL}) / (\text{glucose (mmol/L)} - 3.5)$]. Estimation of serum total cholesterol levels, Estimation of serum HDL-cholesterol immune-enzymatic assay technique, Estimation of serum triglycerides an enzymatic assay, Determination of low-density lipoprotein cholesterol Friedewald's formula, Estimation of serum highly sensitive C-reactive protein (HS-CRP) level was done by immune-enzymatic assay technique described by Buduneli et al., Serum tumor necrotic

factor- α (TNF- α) level was determined by using rat ELISA kits, Measurement of serum malondialdehyde: It was performed by mixing 1 ml PBS (pH 7.0) with 100 mg of tissue using a micro pestle in a microtube until the mix is homogenous, then 20% trichloroacetic acid (TCA) precipitated the protein before being centrifuged, solution of thiobarbituric acid was added to the resulting supernatant once separating it in a different container, then the mix was boiled for ten min in a bath of water to record the absorbance and the ordinary curve was utilized to evaluate the concentration of MDA.¹⁹

Statistical analysis Results were presented as mean \pm SD. Statistical analysis was performed using the statistical package for the social sciences (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.) Repeated variance analysis measures were applied, followed by least significance differences for multiple comparisons. Levels of significance (p) were considered statistically significant when the p-value was less than 0.05.²⁰

Results

Impact nanoparticles (gold & silver) administration on insulin levels in HFD/obese rats the mean values of serum insulin were significant decreases in HFD/obese metformin injected (IIb), then more decreases in HFD/obese metformin, and gold nanoparticles treated (IIc) and more and more decreases in HFD/obese metformin, and silver nanoparticles treated groups (IId). In addition, there were insignificant decreases in serum levels of insulin in HFD/Metformin, gold and silver nanoparticles treated (IIe) in comparison to HFD/obese metformin and silver nanoparticles treated groups (IId) (Figure 1).

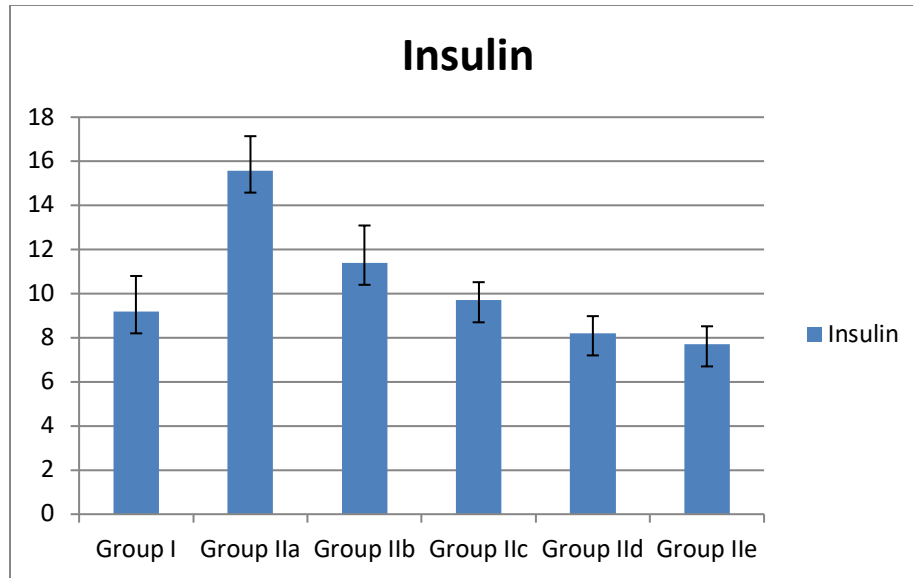


Figure 1: n=10 in each group. Data are represented as mean± SD, Impact nanoparticles (gold & silver) administration on insulin levels HFD/obese metformin injected (IIb), HFD/obese metformin, and gold nanoparticles treated (IIc) and HFD/obese metformin, and silver nanoparticles treated groups (IIId) and HFD/Metformin, gold and silver nanoparticles treated (IIe)

Impact nanoparticles (gold & silver) administration on glucose levels in HFD/obese rats

The mean values of serum levels of glucose were significant decreases in in HFD/obese metformin injected (IIb), HFD/obese metformin, and gold nanoparticles treated (IIc), then near to normal in HFD/obese metformin and silver nanoparticles treated groups (IIId) and HFD/Metformin, gold and silver nanoparticles treated (IIe) (**Figure 11**)

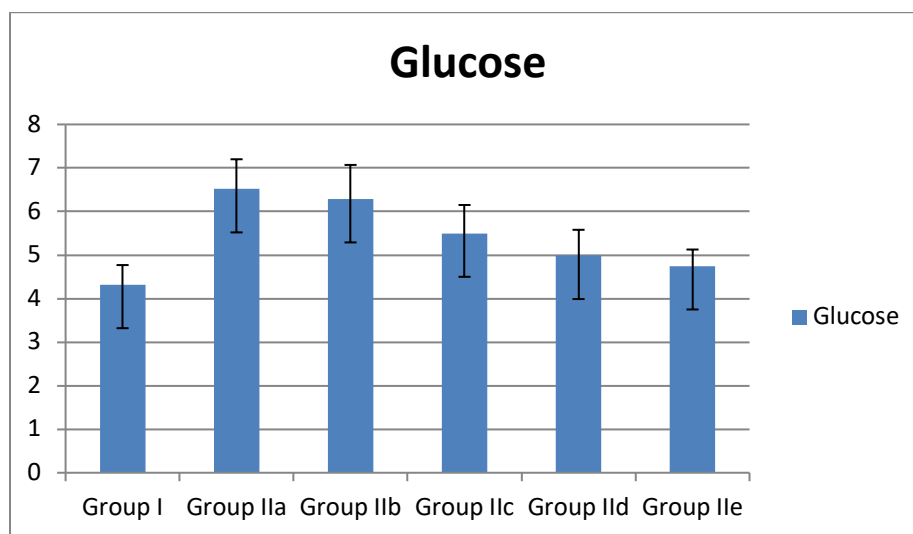


Figure 11: n=10 in each group. Data are represented as mean± SD, Impact nanoparticles (gold & silver) administration on glucose levels HFD/obese metformin injected (IIb), HFD/obese metformin, and gold nanoparticles treated (IIc) and HFD/obese metformin, and silver nanoparticles treated groups (IIId) and HFD/Metformin, gold and silver nanoparticles treated (IIe)

Impact nanoparticles (gold & silver) administration on HOMA levels in HFD/obese rats

The mean values of HOMA IR were significant decreases in HFD/obese metformin injected (IIb) $P < 0.001$, HFD/obese metformin, and gold nanoparticles treated (IIc) $P < 0.001$ and

HFD/obese metformin, and silver nanoparticles treated groups (IIe) $P < 0.001$. In addition, there were significant increases in HOMA B in HFD/Metformin and gold nanoparticles (IIc) $P < 0.05$ in comparison to HFD/obese metformin treated groups (IIb) (Table 1).

Table 1: HOMA levels in all studied groups

	Control (I)	HFD (IIa)	HFD/Metformin treated (IIb)	HFD/Metformin and gold nanoparticles treated (IIc)	HFD/Metformin and silver nanoparticles treated (IIe)	HFD/Metformin, gold and silver nanoparticles treated (IIe)
HOMA IR	$\bar{X} \pm SD$	1.75 ± 0.31 $P < 0.001^{** (a)}$	4.5 ± 0.76 $P < 0.001^{** (b)}$	3.18 ± 0.022 $P < 0.001^{** (c)}$	2.57 ± 0.024 $P < 0.001^{** (d)}$	1.83 ± 0.35 $P = 0.134^{(e)}$
HOMA β	$\bar{X} \pm SD$	36.47 ± 8.7 $P < 0.05^{(a)}$	44.79 ± 7.25 $P < 0.001^{** (b)}$	29.39 ± 5.25 $P < 0.05^{(c)}$	29.59 ± 3.36 $P = 0.10^{(d)}$	33.34 ± 6.05 $P > 0.76^{(e)}$

$n=10$ in each group. Data are represented as mean \pm SD. HDL-C, *, Significant; ** highly significant. ++. (a) P value between group (I) and group (IIa), (b) P value between group (IIa) and group (IIb), (c) P value between group (IIb) and group (IIc), (d) P value between group (IIc) and group (IIe), (e) P value between group (IIe) and group (IIe)

Impact nanoparticles (gold & silver) administration on Lipid profile in HFD/obese rats

There were significant decreases in serum levels of total cholesterol, triglyceride and LDL levels in HFD/obese metformin injected (IIb) ($P < 0.001$), HFD/obese metformin, and gold nanoparticles treated (IIc) ($P < 0.001$, $P < 0.05$, $P < 0.001$, respectively), HFD/obese

metformin and silver nanoparticles treated groups (IIe) ($P < 0.001$). In addition, There were significant increases in serum levels of HDL levels in HFD/obese metformin injected (IIb) $P < 0.05$, HFD/obese metformin, and gold nanoparticles treated (IIc) $P < 0.05$, HFD/obese metformin and silver nanoparticles treated groups (IIe) $P < 0.001$ and HFD/Metformin, gold and silver nanoparticles treated (IIe) $P < 0.05$ (Table 2).

Table 2: Serum changes of lipid profiles in all groups

	Control (I)	HFD (IIa)	HFD/Metformin treated (IIb)	HFD/Metformin and gold nanoparticles treated (IIc)	HFD/Metformin and silver nanoparticles treated (IIe)	HFD/Metformin, gold and silver nanoparticles treated (IIe)
Total cholesterol (mg/dl)	$\bar{X} \pm SD$	173 ± 14.06 $P < 0.001^{** (a)}$	264.2 ± 20.2 $P < 0.001^{** (b)}$	238.9 ± 14.6 $P < 0.001^{** (c)}$	200 ± 6.5 $P < 0.001^{** (d)}$	186.7 ± 6.7 $P = 0.47^{(e)}$
HDL (mg/dl)	$\bar{X} \pm SD$	62.7 ± 8.8 $P < 0.001^{** (a)}$	37.9 ± 5.36 $P < 0.05^{* (b)}$	44.30 ± 5.10 $P < 0.05^{(c)}$	51.5 ± 5.42 $P < 0.001^{** (d)}$	69.6 ± 6.11 $P < 0.05^{(e)}$
Triglycerides (mg/dl)	$\bar{X} \pm SD$	86.9 ± 12.7 $P < 0.001^{** (a)}$	123.1 ± 4.38 $P < 0.001^{** (b)}$	109.7 ± 6.33 $P < 0.001^{** (c)}$	97.4 ± 5.87 $P < 0.001^{** (d)}$	86.7 ± 5.77 $P < 0.001^{** (e)}$
LDL (mg/dl)	$\bar{X} \pm SD$	65.8 ± 14.5 $P < 0.001^{** (a)}$	139.1 ± 9.12 $P < 0.001^{** (b)}$	104.8 ± 5.89 $P < 0.001^{** (c)}$	97.3 ± 4.92 $P < 0.001^{** (d)}$	88.3 ± 4.67 $P = 0.36^{(e)}$

n=10 in each group. Data are represented as mean± SD. HDL-C, *, Significant; ** highly significant. ++. **(a)** P value between group (I) and group (IIa), **(b)** P value between group (IIa) and group (IIb), **(c)** P value between group (IIb) and group (IIc), **(d)** P value between group (IIc) and group (IId), **(e)** P value between group (IId) and group (IIe). Impact nanoparticles (gold & silver) administration on inflammatory markers in HFD/obese rats There were significant decreases in serum levels of CRP, TNF- α and

MDA in HFD/obese metformin injected (IIb) P< 0.001, HFD/obese metformin, and gold nanoparticles treated (IIc) (P< 0.05, P< 0.001 , P< 0.05 respectively) and HFD/obese metformin, and silver nanoparticles treated groups (IId). In addition, there were insignificant decreases in serum levels of CRP, TNF- α and MDA in HFD/Metformin, gold and silver nanoparticles treated (IIe) in comparison to HFD/obese metformin and silver nanoparticles treated groups (IId) (Table 3).

Table 3: Serum changes of inflammatory markers in all groups

		Control (I)	HFD (IIa)	HFD/Metformin treated (IIb)	HFD/Metformin and gold nanoparticles treated (IIc)	HFD/Metformin and silver nanoparticles treated (IId)	HFD/Metformin, gold and silver nanoparticles treated (IIe)
CRP (mg/l)	$\bar{X} \pm$ SD	385.2±.48	481.5±38.33	422.3±44.08	403.5±55.12	383±41.47	373.5±40.88
		P< 0.001 ** ^(a)		P< 0.001 ** ^(b)	P< 0.05* ^(c)	P> 0.05 ^(d)	P=0.61 ^(e)
(TNF- α) (pg/ml)	$\bar{X} \pm$ SD	33±.5.14	60.2±.3.46	47.5±.2.76	40.8±2.47	40.3±.2.98	31.2±.2.44
		P< 0.001 ** ^(a)		P< 0.001 ** ^(b)	P< 0.001** ^(c)	P> 0.05 ^(d)	P< 0.001 ** ^(e)
(MDA)	$\bar{X} \pm$ SD	8.17±.0.28		11.55±.0.93	10.51±.0.72	9.9±.0.59	9.46±.0.56
		P< 0.001 ** ^(a)		P< 0.001 ** ^(b)	P< 0.05* ^(c)	P< 0.05 * ^(d)	P=0.1 ^(e)
(mmol/l)		P< 0.001 ** ^(a)		P< 0.001 ** ^(b)	P< 0.05* ^(c)	P< 0.05 * ^(d)	P=0.1 ^(e)

n=10 in each group. Data are represented as mean± SD. HDL-C, *, Significant; ** highly significant. ++. **(a)** P value between group (I) and group (IIa), **(b)** P value between group (IIa) and group (IIb), **(c)** P value between group (IIb) and group (IIc), **(d)** P value between group (IIc) and group (IId), **(e)** P value between group (IId) and group (IIe).

Discussion

This study is a preliminary step to see how innovative therapies such as nanoparticles can be used in conjunction with existing medication to improve health outcomes. Our present study revealed marked disturbance in the glucose and lipid profile of rats fed with a fat-rich diet manifested by increased serum levels of glucose, insulin, HOMA IR, TC, TG, LDL-C, as well as serum levels of C reactive protein, tumor necrosis factor, Malondialdehyde, and decrease in HDL-C level. These results agree with others who reported that on a high-fat diet, the plasma levels of triglycerides, total cholesterol, LDL-cholesterol, AST, ALT, LDH, and TNF- α are potentially raised.²¹ Also, as expected an

improvement in glycemic control and lipid profile with a decrease in inflammatory markers after metformin treatment was observed. Consistent with **Mariël et al**,²² metformin in the liver cause an overall improvement in lipid metabolism by reducing triglycerides, LDL-C, and total cholesterol. These effects could be mediated via activation of activated protein kinase (AMPK) which then stimulates free fatty acid oxidation, promotes glucose transport, accelerates glycolysis, and inhibits synthesis of both triglyceride and proteins.²³

Moreover, the present results showed that administration of metformin and gold nanoparticles significantly improved dyslipidemia produced by HFD in agreement

with MTF-GNPs (glycation-derived synthesized gold nanoparticles).²⁴ Similarly, Husam M. Edrees *et al.*²⁰ who investigated that AuNPs improved blood glucose level, liver enzymes and pro-inflammatory cytokines causing control of hyperglycemia by administration of gold nanoparticles (10 nm size) through an intraperitoneal injection of 2.5 mg/kg b.w./day for 7 day for 30 albino rats

In the current study, we found significant decrease of serum levels of inflammatory markers and serum levels of malonaldehyde. Our findings are similar to **Karthick *et al.***²⁵ who showed that the serum levels of TNF-, IL-6 and CRP were decreased significantly to the normal level after treatment by gold nanoparticles. Therefore, gold nanoparticles has role in reducing the levels of pro-inflammatory cytokines and suppressing the inflammation.²⁶

Furthermore, administration of AuNPs exhibited an insistent control over the blood glucose level, lipids and serum biochemical profiles in diabetic rats near to the control group provokes their effective role in controlling and increasing the organ functions for better utilization of blood glucose and the possible mechanism for the hypolipidemic effect and hypoglycemic effects of AuNPs might be by insulin-stimulating effect on pancreatic β -cells²⁷

In addition, gold nanoparticles inhibit lipid peroxidation and reactive oxygen species generation, significantly reduced blood glucose levels, and protected the pancreas and liver from oxidative stress-induced injury.²⁸

In addition, our results are supported by the findings that suggest the non-cytotoxic effect of gold nanoparticles, and the ability of gold nanoparticles to reduce the production of reactive oxygen and nitrite species, which do not elicit secretion of pro-inflammatory cytokines TNF- α and IL1- β . The protective effect induced by *C. speciosus* nanoparticles on the prostate of diabetic rats might be directly

mediated through the down-regulation of inflammatory cytokines and the up-regulation of antioxidant activity and indirectly mediated through the anti-hyperglycemic effect through enhancing insulin secretion.²⁹

In contrast to our study **Selim *et al.***, who indicated that there was no difference between the blood glucose level and control level.³⁰ The therapeutic effects that these nanoparticles provide are limited due to the lack of specific protocols dictated by international organizations to evaluate the risks of using these nanoparticles.³¹

The current study revealed hypoglycemic effect after combined treatment of metformin and silver nanoparticles. These findings are in agreement with silver nanoparticles induce a significant reduced blood glucose, higher serum insulin, higher glucokinase activity and higher expression level of insulin.³²

In addition, another study revealed that AgNPs are known for their beneficial effects owing to their anti-diabetic, anti-oxidative, antibacterial, and cytotoxic activities.³³

Side by side to glycemic control, there were improvement of dyslipidemia after combined treatment by metformin and silver nanoparticles.³⁴ In addition the silver nanoparticle was improved lipid profile, energy compensation, oxidative stress and the glycemic in diabetes. In addition, Silver nanoparticles and rosuvastatin have the beneficial effect on hyperlipidemia by improving the lipid profile in the male rats fed on high fat diet.³⁵

In contrast to our result, another study reported that Ag-NPs could exert their toxic effect on the human body through several mechanisms.³⁶ Nano-silver affects the lipid peroxidation and structure of cell membranes, so that, the structural fat of membrane break and this lead to changes in the concentration of plasma lipids.³⁷

In agreement of our study, another study indicated that Nanotechnology in diabetic research is heading towards the novel techniques, which can help in continuous glucose control and improve compliance of diabetic cases.³⁸

Recent studies revealed that nanoparticles might treat diabetic complications such as that study which showed that nanoparticle-based approaches for the treatment of diabetic retinopathy. Furthermore, the nephron-protective effect of NPs reduces the blood glucose level, regulates the renal parameters, decreases the cytokine levels and reduces the mRNA expressions level of different genes related to diabetic nephropathy.³⁹

In agreement with our study, the application of Ag-NPs in animal models resulted in displaying the anti-diabetic effects. AgNPs stimulated uptake of glucose in Hep-2 cells, implying that they have excellent therapeutic and potential anti-diabetic properties. In agreement with our findings, Nanoparticles are known to exploit potential biological properties in producing anti-inflammatory and antibacterial activities. Also they activate cellular mechanisms towards the healing of chronic wounds.⁴⁰

It's important to note, however, that further research would be needed to determine the generalizability of our findings, as well as to assess the safety and potential adverse effects of these interventions as proposed for example by **Liao C et al.**⁴¹ who reported that AgNPs are toxic to several human cell lines, including human bronchial epithelial cells, human umbilical vein endothelial cells, red blood cells, human peripheral blood mononuclear cells, immortal human keratinocytes and liver cells. AgNPs induce a dose-, size- and time-dependent cytotoxicity.

Conclusion

The results of the current study propose that treatment with gold and/or silver nanoparticles

for 28 days in rats with induced diabetes improved blood glucose level, insulin resistance, and insulin sensitivity. In addition, combining nanoparticles and metformin treatment could be effective in glucose monitoring and dyslipidemia. We concluded that nanoparticles could have anti-diabetic and anti-inflammatory effect.

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