RESEARCH PAPER

Silver nanoparticles modulate high fat high carbohydrate diet induced metabolic changes in rats via reducing lipid peroxidation, PDGF-β and insulin resistance

Mahmoud Ashri [1], Nashwa Abu-Elsaad [1], Tarek Ibrahim [1]*

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura Egypt*

ABSTRACT

Objective(s): Non-alcoholic fatty liver disease (NAFLD) is strongly linked with insulin resistance and type-2 diabetes through various metabolic changes. The current study was designed to investigate the modulatory effects of silver nanoparticles (AgNPs) on metabolic and inflammatory changes activated during NAFLD.

Materials and Methods: Three doses of AgNPs (100, 150, and 200 µg/kg/day for 4 weeks) were tested in a high-fat high carbohydrate diet (HFHCD) induced NAFLD model in rats.

Results: Sinificant (P<0.05) improvement in dyslipidemia, hyperglycemia, and insulin levels by AgNPs was observed and more notably in the group that received 200 µg/kg/day AgNPs. Acute phase inflammatory protein C-reactive protein and monocytes chemoattractant protein-1 were significantly (P<0.05) lowered by AgNPs. Inline, lipid peroxidation and PDGF-β levels were significantly (P<0.05) reduced in groups that received different doses of AgNPs. Furthermore, AgNPs especially in the large dose (200 µg/kg/day) significantly decreased (P<0.05) the measured level of the inflammatory cytokines (IL-1β, IL-6, and TNF-α) compared with the HFHCD group level.

Conclusion: Collectively, results propose the ability of AgNPs to modulate metabolic changes accompanying NAFLD through reducing lipid peroxidation and targeting inflammatory cytokines mediating insulin resistance.

Keywords: *Fatty liver; Insulin resistance; Nanoparticles; Silver*

How to cite this article

Ashri M, Abu-Elsaad N, Ibrahim T. Silver nanoparticles modulate high fat high carbohydrate diet induced metabolic changes in rats via reducing lipid peroxidation, PDGF-β and insulin resistance. Nanomed J. 2022; 9(4): 296-306. DOI: 10.22038/NMJ.2022.67356.1712

INTRODUCTION

There are two types of biological dysfunctions that frame type-2 diabetes (T2D), namely β-cell dysfunction and insulin receptors dysfunction [1]. Beta cell dysfunction may be due to genetic factors or acquired factors such as obesity, an unbalanced diet such as high fat-high carbohydrate diet (HFHCD), or acquired inflammatory insult that destructs β-cells [2].

Nonalcoholic fatty liver disease (NAFLD) is strongly associated with hepatic insulin resistance and is a major factor in T2D pathogenesis and nonalcoholic steatohepatitis as well. The accumulated fat metabolites like long-chain acyl-CoA, diacylglycerol, and ceramides lead to activation of protein kinases, and in turn an increased rate of insulin receptor phosphorylation, then their deactivation due to signaling inhibition [3].

The prevalence of NAFLD is 4–6 times higher in obese than in normal individuals [4]. Obesity-induced hormonal imbalance (stimulated leptin, glucagon activity, and inhibited adiponectin activity) plays a crucial role in insulin receptor hypofunction. In addition to such hormonal imbalance, inflammatory cytokines released from adipose tissue also have an important impact on obesity-induced insulin receptor hypofunction. So far, pharmacological agents that tend to ameliorate NAFLD have shown limited success [5].

Nanotechnology is concerned with follow-up, reformation, reconstruction, and managing human physiological systems at the cell level by using materials engineered at the molecular level

* *Corresponding author: Email: ashrimahmoud22@gmail.com*

Note. This manuscript was submitted on July 10, 2022; approved on September 21, 2022

[6]. The application of nanotechnology in medicine has shown promising benefits, especially in cancer research [7-9]. In experimental studies, metals like silver and gold were found to have a potential therapeutic value in some chronic diseases especially diabetes [10]. An acute toxicity study done by Arumugam *et al*. revealed no acute toxic effects of silver nanoparticles (AgNPs) in addition to zero % mortality during the whole period of the experiment [11]. The absence of a "concentration effect" on the adsorption, distribution, metabolism, and excretion rates of inhaled AgNPs is considered an additional advantage [12]. Furthermore, AgNPs are easily eliminated from the body as previously demonstrated by Williams *et al* [13].

AgNPs show high antioxidant activity and remain in circulation in reasonable concentrations for a significant time [14,15]. Many therapeutic benefits of AgNPs have been studied including anticancer and antimicrobial benefits [16,17]. Additionally, AgNPs alleviate chemically induced colitis, proposing their plausible use in inflammatory diseases [18]. Various *in vitro* studies showed antidiabetic activity of AgNPs [19,20]. *In vivo*, injecting AgNPs into the peritoneal cavity can protect rats against dextrose-induced diabetes and markedly improve metabolic parameters [21]. The current study was designed to test the effect of AgNPs on HFHCD-induced NAFLD and the ability of nanoparticles to control accompanying metabolic changes and their impact on T2D.

Experimental section

Animals

Forty-five male Wistar rats weighing (150–180 g each) were used. They were purchased from VCSERA (Egypt), and were allowed water and food freely for the period of acclimatization (two weeks). The experimental work follows the guidelines and ethical principles for animal care and use approved by the scientific laboratory research ethics committee, faculty of pharmacy, Mansoura university.

Drugs and chemicals

Silver nanoparticle solution was obtained from Lotus Middle East Pharma (Egypt). According to the manufacturer's datasheet, the solution contains AgNPs of 60 nm in diameter dispersed in sterile water at a concentration of 1 g/L. A stock solution of 1 ml was diluted with 9 ml sterile water to obtain a working solution of 100 µg silver nanoparticles per 1 ml sterile water (10% w/v).

As described by the manufacturer, AgNPs were prepared as follows: 0.2 g sodium hydroxide was dissolved in a small amount of distilled water. 1 g of rice starch was added gradually to the sodium hydroxide solution under stirring within 5 min. The solution was completed to 100 ml with distilled water till complete solubilization of starch. The resulting sodium starchate solution was heated to 60 °C and the pH of the solution was adjusted to 11.5 by using a 10 M aqueous solution of sodium hydroxide. 1.56 g silver nitrate was dissolved in 100 ml distilled water. 1 ml of the silver nitrate solution was added dropwise to the sodium starchate solution at 60 °C at a pH of 11.5 under vigorous stirring for 30 min. During the addition of the sodium starchate solution the color gradually turned from an obscure white color to transparent yellow color indicating the formation of silver nanoparticles. The commercial AgNPs solution used in the study was previously characterized and optimized by [22].

Experimental design

Rats were divided randomly into five groups (9 rats each): Control group: fed standard rat chow (150 g/kg/day) for 16 weeks and 0.5 ml/kg normal saline orally at the beginning of week 13. HFHCD group: allowed free access to HFHCD for 16 weeks and 0.5 ml/kg normal saline orally at the beginning of week 13. AgNP100 group: received HFHCD freely for 16 weeks plus 100 µg/kg/day of AgNPs as 10% w/v solution (1 ml/kg/day) at the beginning of week 13 orally with oral gavage for 4 weeks. AgNP150 group: received HFHCD plus 150 µg/kg/day AgNPs solution (1.5 ml/kg/day of working solution) as described. AgNP200: received HFHCD plus 200 µg/kg/day AgNPs solution (2 ml/kg/day of working solution) as described.

METHODS

Induction of NFLD and T2D

Rats received HFHCD *ad libitum* [components: beef tallow 300, molasses 200, and standard rat chow 500 (g/kg diet)] daily for 16 weeks as described by Abu-Elsaad *et al* [23]. Twenty-four hours following the last AgNPs dose, rats were anesthetized with thiopental sodium IP injection (50 mg/kg), chests were opened, blood was collected through cardiac puncture and centrifuged at 3000 rpm for ten minutes for serum separation. The liver was isolated and weighed, and the liver homogenate was prepared manually by grinding 100 mg wet tissue in 1 ml ice-cooled phosphate buffer saline (pH 7.4) followed by centrifugation at

4000 rpm, -4 °C. The supernatant was separated and used for measuring liver homogenate (10% w/v) parameters.

Measurement of lipid profile

Serum levels of total cholesterol (TC), low-density lipoproteins (LDL-C), triglycerides (TG), and high-density lipoproteins (HDL-C) were measured spectrophotometrically (Spinreact Co., Spain) according to the instructions of the manufacturer.

Measurement of serum glucose and insulin levels

Blood glucose level was measured using a glucose assay colorimetric kit supplied by Cell Biolabs, Inc. (San Diego, USA) at 560 nm. Serum insulin level was assigned using the ELISA technique as directed by the manufacturer (eBioscience, San Diego, USA).

Measurement of lipid peroxidation

The liver content of malondialdehyde (MDA) was measured in liver homogenate as previously described by Ohkawa *et al* [24]. Samples (0.2 ml liver homogenate) were mixed with thiobarbituric acid at acidic pH for 30 min at 95 °C, and the produced color absorbance was measured at 534 nm.

Measurement of C-reactive protein (CRP) and monocyte chemoattractant protein (MCP)-1

CRP serum level and liver content of MCP-1 were measured using the ELISA technique as directed by the manufacturer (ThermoFisher Scientific Inc., MA, USA).

Measurement of inflammatory cytokines and growth factors

Liver expression of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and platelet-derived growth factor (PDGF)-β were determined using ELISA kits (ThermoFisher Scientific Inc., MA, USA).

RESULTS

Levels of serum TC, TG, and LDL-C (Figures 1a, 1b, and 1c, respectively) were significantly increased as a result of feeding rats HFHCD for 16 weeks compared with their levels in the control group ($P<0.001$). On the other hand, the serum level of HDL-C (Figure 1d) significantly decreased in the HFHCD group ($P<0.001$).

Oral administration of AgNPs in the three tested

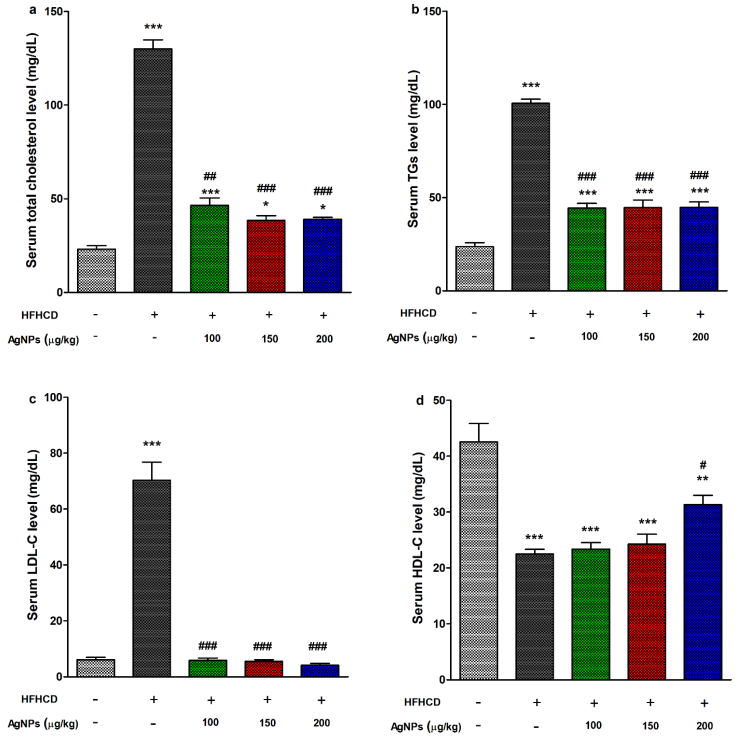

Fig. 1. Effect of silver nanoparticles (AgNPs) on lipid profile serum biomarkers. (a) total cholesterol, (b) triglycerides (TGs), (c) low-density lipoprotein (LDL-C), (d) high-density lipoprotein (HDL-C) in high-fat high carbohydrate diet (HFHCD) induced insulin resistance model in rats. Significance: *, *** $P<0.05$, 0.001, respectively compared with the control group; #, ##, ### $P<0.05$, 0.01, 0.001, respectively compared with HFHCD group

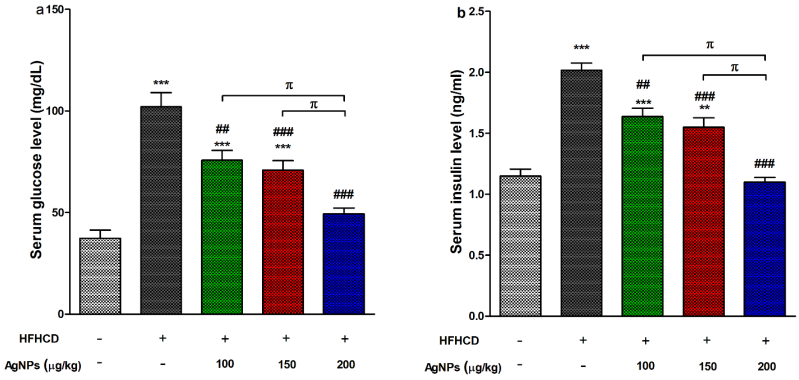

Fig. 2. Effect of silver nanoparticles (AgNPs) on (a) serum glucose and (b) serum insulin in high-fat high carbohydrate diet (HFHCD) induced insulin resistance model in rats. Signif-icance: **, *** *P*<0.01, 0.001, respectively compared with control group; ##, ###, *P*<0.01, 0.001, respectively compared with HFHCD group; π *P*<0.05

doses for the last 4 weeks resulted in a significant (*P*<0.01) decrease in serum level of TC compared with the HFHCD group but remained significantly higher (*P*<0.05) compared with the control group. Serum TG was significantly (*P*<0.001) low in groups that received AgNPs compared with the HFHC group and significantly higher than the control group level (*P*<0.05). Serum level of LDL-C was significantly (*P*<0.001) lowered by AgNPs and nearly reached normal levels in the control group. HDL-C level increased significantly compared with its level in the HFHCD group (*P*<0.05) only in the group that received 200 *μg*/kg AgNPs. Its level remained significantly less than the control group level (*P*<0.01) in all groups that received AgNPs.

Feeding rats HFHCD for sixteen weeks resulted in a significant increase in serum glucose levels compared with rats that received standard chow (*P*<0.001). Administration of AgNPs from the 13th week significantly reduced serum glucose levels with the highest reduction in the group that received the higher dose of AgNPs (200 *μg*/kg) (*P*<0.001), which showed no significant difference compared with the control group. However, serum glucose level remained (*P*<0.001) higher in groups that received 100 or 150 *μg*/kg AgNPs than its level in the control group (Figure 2a). The glucose level in the group that received 200 *μg*/kg AgNPs was significantly (*P*<0.05, 0.01) lower than in groups that received 100 or 150 *μg*/kg AgNPs, respectively.

Similar effects of AgNPs were observed on serum insulin levels (Figure 2b). Rats who received HFHCD for 16 weeks showed about a 2-fold increase in serum insulin level compared with the control group (*P*<0.001). Administration of AgNPs significantly decreased serum insulin compared with its elevated level in the HFHCD group (*P*<0.01). Improvement in insulin level was best observed in the AgNP200 group and reached the control group level. Serum insulin was significantly (*P*<0.001) less in AgNP200 group compared with AgNP100 and AgNP150.

Lipid peroxidation biomarker MDA was significantly (*P*<0.001) lowered by tested AgNPs doses in a dose-dependent manner (Figure 3a). In AgNP1.5 and AgNP200 groups, MDA level was significantly (*P*<0.001, 0.05) lower compared with HFHCD group and AgNP100, respectively.

The level of the acute inflammation phase protein CRP was significantly elevated by feeding rats HFHCD (*P*<0.001). All tested doses significantly (*P*<0.001) decreased CRP serum levels compared with the HFHCD group and showed no significant difference from the control group (Figure 3b).

Rats fed HFHCD showed elevated MCP-1 content in hepatic tissue compared with those fed standard show (*P*<0.001). In AgNP100 and AgNP150 groups, MCP-1 expression was significantly (0.001) reduced compared with the HFHC diet group but remained significantly higher (*P*<0.001) than in the control group. Highest significant (*P*<0.001) reduction in MCP-1 content was observed in AgNP200 group (Figure 3c) compared with HFHC diet, AgNP100, and AgNP150 groups.

Liver expression of both IL-1β and IL-6 was increased (*P*<0.001) significantly by HFHCD compared with the control group (Figures 4a and 4b, respectively). Groups that received AgNPs solution showed a significantly (*P*<0.001) low hepatic level of these cytokines compared with the HFHCD group. In the AgNP200 group, both cytokines levels were significantly (*P*<0.05) less

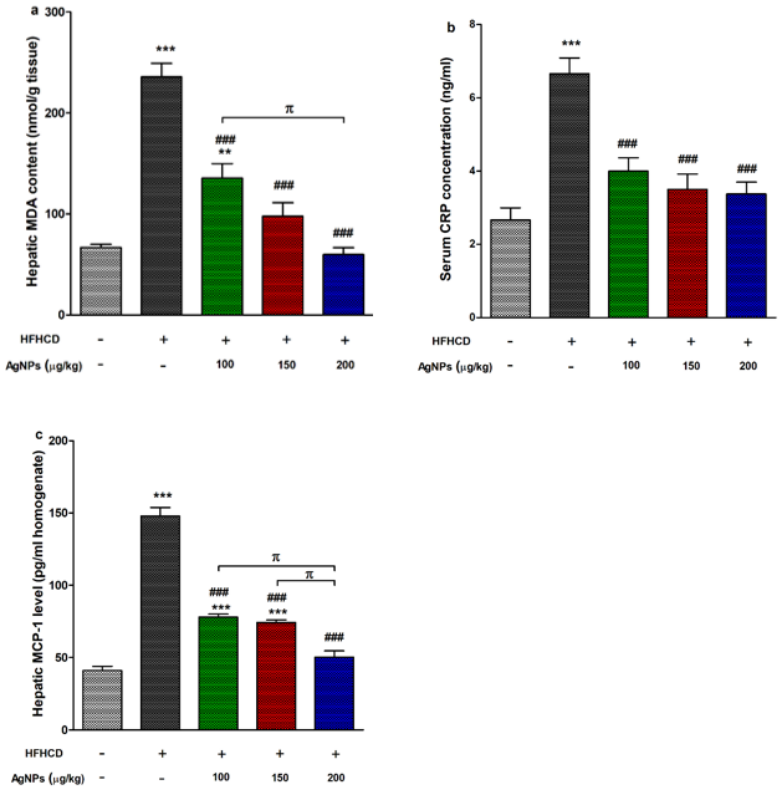

Fig. 3. Effect of silver nanoparticles (AgNPs) on (a) hepatic malondialdehyde (MDA), (b) serum C-reactive protein (CRP), (c) hepatic monocyte chemoattractant protein-1 (MCP-1) in high-fat high carbohydrate diet (HFHCD) induced insulin resistance model in rats. Signifi-cance: **, *** P<0.01, 0.001, respectively compared with control group; ### P<0.001 com-pared with HFHCD group; π P<0.05

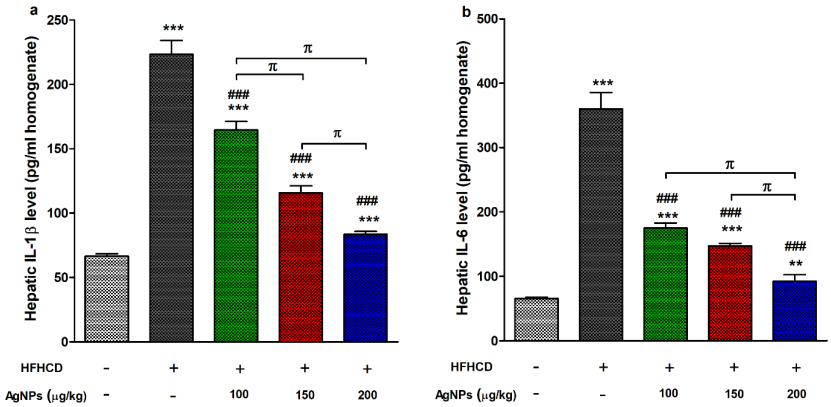

Fig. 4. Effect of silver nanoparticles (AgNPs) on hepatic (a) interleukin (IL)-1β and (b) IL-6 in high-fat high carbohydrate diet (HFHCD) induced insulin resistance model in rats. Significance: **, *** P<0.01, 0.001, respectively compared with control group; ### P<0.001 compared with HFHCD group; π P<0.05

than levels in AgNP100 and AgNP150 groups.

Similarly, TNF-α expression was significantly (P<0.001) decreased in a dose-dependent manner by AgNPs tested doses compared with its expression in HFHCD (Figure 5a). However, the expression of TNF-α in AgNPs groups was still significantly (P<0.001) higher than in the control group. A significant difference (P<0.05) in TNF-α concentration was observed between AgNPs groups with the lowest concentration in the AgNP200 group.

Group that received 2 ml/kg AgNPs 10% w/v solution showed a significant (P<0.001) decrease in PDGF-β level compared with HFHCD, AgNP100,

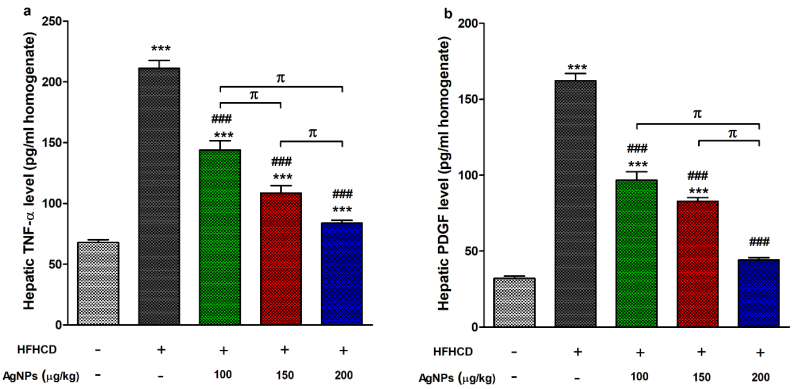

Fig. 5. Effect of silver nanoparticles (AgNPs) on hepatic (a) tumor necrosis factor (TNF)-α and (b) platelet-derived growth factor (PDGF) in high-fat high carbohydrate diet (HFHCD) induced insulin resistance model in rats. Significance: *** P<0.001 com-pared with control group; ### P<0.001 compared with HFHCD group; π P<0.05

and AgNP150 groups. Smaller doses of AgNPs also decreased growth factor level, but they remained significantly (P<0.001) higher than the control group level (Figure 5b).

DISCUSSION

In the current study, rats fed HFHCD showed elevated serum glucose levels and hyperinsulinemia. Besides, significant dyslipidemia was observed in this group confirming metabolic changes accompanying developed insulin resistance. Oral administration of AgNPs resulted in improving lipid profile biomarkers, halting lipid peroxidation, regulating insulin and blood glucose levels in addition to modulating inflammatory cytokines.

The pathogenesis involved in NAFLD development is complex and multifactorial. The “two hit” theory is the best to describe pathogenesis [25]. The first hit after the intake of high dietary fat is steatosis which liberates free fatty acids from adipose tissue and induces hepatic lipid secretion inadequately with insulin resistance [26]. Afterward, the second hit involves oxidative stress induction, lipid peroxidation, and inflammatory cytokine release leading to steatohepatitis [25].

One of the accepted assumptions about the mechanisms behind B-cell dysfunction/insulin receptor hypofunction (insulin resistance) and the consequently impaired glucose tolerance is the increased production of oxygen species [27]. Moreover, such increased oxygen species may be the underlying mechanism of vascular injury either macrovascular or microvascular as the most dangerous complications of T2D [28]. Accordingly, using antioxidant therapies would be beneficial for diabetic patients as management or even a prophylactic therapy for high-risk patients.

The limited absorption and ineffective distribution of the currently available oral antioxidants may account for their low efficiency. Metallic nanoparticles (MNPs) based therapy has many benefits as an antioxidant therapy which include higher bioavailability, less dosing frequency, good chance for passing through the drastic conditions in the stomach, in addition to lower side effects and more site specificity [29]. Some studies reported a potential increase in intracellular oxidative stress on using certain MNPs due to mitochondrial damage [30]. In the current study, AgNPs decreased lipid peroxidation as indicated by low MDA hepatic content supporting its antioxidant capability. In addition, AgNPs can act as scavengers for reactive oxygen species accounting for another proposed antioxidant mechanism [31].

Silver nanoparticles have a wide range of particle sizes varying from 2 nm to several 100 nm. They are among the smallest MNPs available for medical use with very low toxic potentialities. The toxicity of AgNPs *in vivo* depends on particle size and complexity of biological systems which affect particles’ cellular uptake and accumulation [32]. An acute toxicity study on mice showed various histopathological changes as hepatic focal necrosis and spleen congestion after administration of smaller-sized AgNPs (10 nm in diameter) while these changes were not evident with larger particles (60 and 100 nm) [33]. On the contrary, *in vitro* studies reported that cytotoxicity of AgNPs occurs obviously with the nanoparticles of size more than 100 nm where most affected tissues

include immune cells, lung epithelial cells, skin, and neurons [34]. Also, AgNPs have the advantages of a large surface area and more ability to stimulate different ligands' functions [35, 36]. The use of AgNPs as a potent antioxidant may represent a promising treatment for insulin resistance and NFLD induced by HFHCD. Understanding the mechanisms employed by AgNPs for controlling glycemic status is a cornerstone for its application in managing metabolic changes in T2D patients. The present study was designed as a trial to investigate some of these mechanisms.

Some investigators suggested that waving blood glucose levels may be the underlying cause of the increased oxidative stress more than chronic hyperglycemia [37, 38]. Studying the effect of unsteady vs. steady glycemic state in cells isolated from human kidneys revealed increased production of TGF-β and IGFBP-3 in the medium that was exposed to the unsteady glucose concentration more than in that which was exposed to a steady elevated glucose level [39].

A new strategy for controlling the blood glucose level in T2D without dietary intervention would be a breakthrough in the dilemma of controlling the glycemic status. Despite the valuable advantages of therapeutic lifestyle modification, it may be problematic for many patients to follow the recommendations needed for such type of therapy because of the natural tendency for laziness and overeating in the modern world [40]. Our study supports the assumption of AgNPs' validity in improving the hepatic role in controlling lipid and carbohydrate metabolism in rats suffering from insulin resistance induced by HFHCD received chronically.

Feeding rats with HFHCD was accompanied by an increase in liver MDA content. Accumulated liver fats stimulate the expression of NADPH oxidase which in turn increases the production of oxygen free radicals [41]. The dysregulated hepatic redox state results in 1) hepatic mitochondrial damage leading to inhibition of the mitochondrial biogenesis and decreased utilization of lipids by the mitochondria and dyslipidemia as the net result [42,43] and 2) stimulation of monocytes and neutrophil infiltration into the liver to phagocyte the excess production of oxygen species and streaming of hepatic proinflammatory cytokines specially IL-1β, IL-6, PDGF, and TNF-α by the resident and recruited macrophages and neutrophils. These proinflammatory cytokines aggravate the dysregulation of mitochondrial biogenesis and dyslipidemia [44,45]

Administration of AgNPs reduced lipid peroxidation and measured inflammatory cytokines and CRP levels indicating an improvement in the inflammatory status. In addition, AgNPs reduced MCP-1 hepatic content which plays a critical role in monocytes and neutrophils' recruitment into the liver [46, 47]. Halting the activity of MCP-1 and immune cell recruitment limits the streaming of proinflammatory cytokines in liver tissue.

Another supporting observation is the significant normalization of insulin blood levels in animals fed HFHCD concurrently with AgNPs. Induction of NAFLD in rats *via* HFHCD leads to suppression of the ability of hepatic insulin to induce glycogenesis [48]. This diminished ability of hepatic insulin to suppress hepatic glucose production is attributed to the increased hepatic oxidative stress, liver fat accumulation, and subsequent liver inflammation [49,50]. It also leads to hyperinsulinemia as a direct result of increased hepatic glucose production [51]. The decreased hyperglycemia in AgNPs received groups may indicate the restoration of the hepatic role in controlling carbohydrate metabolism.

The increased hepatic production of pro-inflammatory cytokines, such as TNF-α and IL-6 because of overnutrition with HFHCD are among the critical links between fatty liver and insulin resistance [52]. TNF-α is an adipose tissue-derived pro-inflammatory cytokine. It was found that insulin sensitivity is inversely proportional to the increased activity of TNF-α and thus targeting TNF-α signaling could be a successful therapeutic approach for management of insulin resistance [53]. Reactive oxygen species are the crucial regulators of TNF-α signaling [54, 55] and they sensitize liver cells to TNF-α inflammatory and apoptotic effects. Accordingly, the antioxidant effect of AgNPs is delegated to be the modulator of the TNF-α signaling pathway in liver cells and hence decreased NAFLD-induced insulin resistance in the treated group.

Many investigators mentioned that the increased activity of IL-6 in the liver is directly proportional to insulin resistance and treatment with IL-6 antibodies increases hepatic insulin sensitivity [56-58]. However, the role of IL-6 in NAFLD is a matter of debate. It was reported that IL-6 improves mitochondrial biogenesis and decreases lipid disturbance [59]. Results of

the present study showed a reducing effect of AgNPs on hepatic IL-6 activity and are strongly accompanied by a significant improvement of hepatic insulin resistance as indicated by ameliorating hyperinsulinemia in the treated groups.

CONCLUSION

From the obtained results a potential ability of AgNPs to modulate metabolic changes accompanying NAFLD can be proposed. Silver nanoparticles showed an antioxidant effect that limits lipid peroxidation and in turn can halt initial injury pathways. In addition, they decrease inflammatory cell infiltration, reducing inflammatory cytokines involved in insulin resistance and progression of NAFLD. Further studies on different models of insulin resistance and diabetic metabolic changes are recommended.

ACKNOWLEDGMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Olefsky JM, Nolan JJ. Insulin resistance and non-insulin-dependent diabetes mellitus: cellular and molecular mechanisms. Am J Clin Nutr. 1995;61(4):980S-986S.
2. Kahn SE, The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia 2003;46(1):3-19.
3. Milanski M, Degasperi G, Coope A, Morari J, Denis R, Cintra DE, Tsukumo DM, Anhe G, Amaral ME, Takahashi HK, Curi R. Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity. J Neurosci 2009;29(2):359-370.
4. Dunn W, Schwimmer JB. The obesity epidemic and nonalcoholic fatty liver disease in children. Curr Gastroenterol Rep 2008;10(1):67-72.
5. Perry RJ, Zhang D, Zhang XM, Boyer JL, Shulman GI. Controlled-release mitochondrial protonophore reverses diabetes and steatohepatitis in rats. Science. 2015;347(6227):1253-1256.
6. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol 2018;9(1):1050-1074.
7. Khuroo T, Atifa U, Iqbal Z. Oral Formulation of Paclitaxel and Erlotinib Polymeric Nanoparticles: A Potential Combination to Treat Breast Cancer. Biomed J Sci Technol Res 2020;31(4):24338-24340.
8. Anzar N, Mirza MA, Anwer K, Khuroo T, Alshetaili AS, Alshahrani SM, Meena J, Hasan N, Talegaonkar S, Panda AK, Iqbal Z. Preparation, evaluation and pharmacokinetic studies of spray dried PLGA polymeric submicron particles of simvastatin for the effective treatment of breast cancer. J Mol Liq 2018;249:609-16.
9. Padhi S, Mirza MA, Verma D, Khuroo T, Panda AK, Talegaonkar S, Khar RK, Iqbal Z. Revisiting the nanoformulation design approach for effective delivery of topotecan in its stable form: An appraisal of its in vitro Behavior and tumor amelioration potential. Drug Deliv 2016;23(8):2827-2837.
10. Shaheen TI, El-Naggar ME, Hussein JS, El-Bana M, Emara E, El-Khayat Z, Fouda MM, Ebaid H, Hebeish A. Antidiabetic assessment; in vivo study of gold and core-shell silver-gold nanoparticles on streptozotocin-induced diabetic rats. Biomed Pharmacother 2016;83:865-875.
11. Sengottaiyan A, Aravinthan A, Sudhakar C, Selvam K, Srinivasan P, Govarthanan M, Manoharan K, Selvankumar T. Synthesis and characterization of Solanum nigrum-mediated silver nanoparticles and its protective effect on alloxan-induced diabetic rats. J Nanostructure Chem 2016; 6(1):41-48.
12. Sung JH, Ji JH, Park JD, Yoon JU, Kim DS, Jeon KS, Song MY, Jeong J, Han BS, Han JH, Chung YH. Subchronic inhalation toxicity of silver nanoparticles. Toxicol Sci 2009;108(2):452-461. Williams K, Milner J, Boudreau MD, Gokulan K, Cerniglia CE, Khare S. Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of Sprague-Dawley rats. Nanotoxicology 2015;9(3):279-789.
13. Mittal AK, Bhaumik J, Kumar S, Banerjee UC. Biosynthesis of silver nanoparticles: elucidation of prospective mechanism and therapeutic

potential. J Colloid Interface Sci 2014;415:39-47.
14. Dziendzikowska K, Gromadzka-Ostrowska J, Lankoff A, Oczkowski M, Krawczyńska A, Chwastowska J, Sadowska-Bratek M, Chajduk E, Wojewodzka M, Dušinská M, Kruszewski M. Time-dependent biodistribution and excretion of silver nanoparticles in male Wistar rats. J Appl Toxicol 2012;32(11):920-928.
15. Begum SR, Rao DM, Reddy PD. Role of Green Route Synthesized Silver Nanoparticles in Medicinal Applications with Special Reference to Cancer Therapy. Biosci Biotechnol Res Asia 2018; 15(4):783-790.
16. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, Galdiero M. Silver nanoparticles as potential antibacterial agents. Molecules 2015; 20(5):8856-8874.
17. Krajewska JB, Długosz O, Sałaga M, Banach M, Fichna J. Silver nanoparticles based on blackcurrant extract show potent anti-inflammatory effect in vitro and in DSS-induced colitis in mice. Int J Pharm 2020;585:119549.
18. Jini D, Sharmila S. Green synthesis of silver nanoparticles from Allium cepa and its in vitro antidiabetic activity. Mater. Today: Proc. 2020;22:432-438.
19. Popli D, Anil V, Subramanyam AB, MN N, VR R, Rao SN, Rai RV, Govindappa M. Endophyte fungi, Cladosporium species-mediated synthesis of silver nanoparticles possessing in vitro antioxidant, anti-diabetic and anti-Alzheimer activity. Artif Cells Nanomed Biotech 2018;46:676-683.
20. Birudu RB, Naik MJ, Janardhan M. Ethanolic extract of Passiflora foetida and silver nanoparticles on carbohydrate metabolic enzymes of dextrose induced diabetic rats. J Biochem Biopharm Biomed Sci 2015;1(1):12-19.
21. Mosa WF, El-Shehawi AM, Mackled MI, Salem MZ, Ghareeb RY, Hafez EE, Behiry SI, Abdelsalam NR. Productivity performance of peach trees, insecticidal and antibacterial bioactivities of leaf extracts as affected by nanofertilizers foliar application. Sci Rep 2021;11(1):1-9.
22. Abu-Elsaad N, El-Karef A. Protection against nonalcoholic steatohepatitis through targeting IL-18 and IL-1alpha by luteolin. Pharmacol Rep 2019;71(4): 688-694.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95(2):351-358.
24. Fang YL, Chen H, Wang CL, Liang L. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: From "two hit theory" to "multiple hit model". World J Gastroenterol 2018;24(27):2974.
25. Te Sligte K, Bourass I, Sels JP, Driessen A, Stockbrűgger RW, Koek GH. Non-alcoholic steatohepatitis: review of a growing medical problem. Eur J Intern Med 2004;15(1): 10-21
26. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol 2004;24(5):816-823.
27. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414(6865):813-820.
28. Rai VK, Mishra N, Agrawal AK, Jain S, Yadav NP. Novel drug delivery system: an immense hope for diabetics. Drug Deliv 2016;23(7):2371-2390
29. Soenen SJ, Rivera-Gil P, Montenegro JM, Parak WJ, De Smedt SC, Braeckmans K. Cellular toxicity of inorganic nanoparticles: common aspects and guidelines for improved nanotoxicity evaluation. Nano today 2011;6(5):446-465.
30. Annu M, Ahmed S, Kaur G, Sharma P, Singh S, Ikram S. Evaluation of the antioxidant, antibacterial and anticancer (lung cancer cell line A549) activity of Punica granatum mediated silver nanoparticles. Toxicology research. 2018 Sep 1;7(5):923-30.
31. Vazquez-Muñoz R, Borrego B, Juárez-Moreno K, García-García M, Morales JD, Bogdanchikova N, Huerta-Saquero A. Toxicity of silver nanoparticles in biological systems: does the complexity of biological systems matter? Toxicol Lett 2017;276:11-20.
32. Cho YM, Mizuta Y, Akagi JI, Toyoda T, Sone M, Ogawa K. Size-dependent acute toxicity of silver nanoparticles in mice. Journal of toxicologic pathology. 2018;31(1):73-80.
33. Kim TH, Kim M, Park HS, Shin US, Gong MS, Kim HW. Size-dependent cellular toxicity of silver nanoparticles. J Biomed Mater Res A 2012;100(4):1033-1043.
34. Krutyakov YA, Kudrinskiy AA, Olenin AY, Lisichkin GV. Synthesis and properties of silver nanoparticles: advances and prospects. Russ Chem Rev 2008;77(3):233.

35. Guo JZ, Cui H, Zhou W, Wang W. Ag nanoparticle-catalyzed chemiluminescent reaction between luminol and hydrogen peroxide. J Photochem Photobiol A 2008;193(2-3):89-96
36. Hirsch IB, Brownlee M. Should minimal blood glucose variability become the gold standard of glycemic control? J Diabetes Complicat 2005;19(3):178-181
37. Hirsch IB. Intensifying insulin therapy in patients with type 2 diabetes mellitus. Am J Med 2005;118(5):21-26
38. Jones SC, Saunders HJ, Qi W, Pollock CA. Intermittent high glucose enhances cell growth and collagen synthesis in cultured human tubulointerstitial cells. Diabetologia 1999;42(9):1113-1119
39. Shomali M. Diabetes treatment in 2025: can scientific advances keep pace with prevalence? Ther Adv Endocrinol 2012;3(5):163-173
40. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Investig 2017;114(12):1752-1761
41. Simoneau JA, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. FASEB J 1999;13(14):2051-2060
42. Lee HM, Kim JJ, Kim HJ, Shong M, Ku BJ, Jo EK. Upregulated NLRP3 inflammasome activation in patients with type 2 diabetes. Diabetes. 2013;62(1):194-204
43. Meng XF, Wang XL, Tian XJ, Yang ZH, Chu GP, Zhang J, Li M, Shi J, Zhang C. Nod-like receptor protein 1 inflammasome mediates neuron injury under high glucose. Mol Neurobiol 2014;49(2):673-684
44. Koenen TB, Stienstra R, Van Tits LJ, De Graaf J, Stalenhoef AF, Joosten LA, Tack CJ, Netea MG. Hyperglycemia activates caspase-1 and TXNIP-mediated IL-1β transcription in human adipose tissue. Diabetes. 2011;60(2):517-524
45. Serbina NV, Jia T, Hohl TM, Pamer EG. Monocyte-mediated defense against microbial pathogens. Annu Rev Immunol 2008;26:421-452
46. Weber C, Belge KU, Von Hundelshausen P, Draude G, Steppich B, Mack M, Frankenberger M, Weber KS, Ziegler-Heitbrock HW. Differential chemokine receptor expression and function in human monocyte subpopulations. J Leukoc Biol 2000;67(5):699-704
47. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ, Shulman GI. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem 2004;279(31):32345-32353
48. Zhang XQ, Xu CF, Yu CH, Chen WX, Li YM. Role of endoplasmic reticulum stress in the pathogenesis of nonalcoholic fatty liver disease. WJG 2014;20(7):1768
49. Gross B, Pawlak M, Lefebvre P, Staels B. PPARs in obesity induced T2DM, dyslipidaemia and NAFLD. Nat Rev Endocrinol 2017;13(1):36-49
50. McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. Science 1992;258(5083):766-770
51. Sharma M, Vikram NK, Misra A, Bhatt S, Tarique M, Parray HA, Pandey RM, Luthra K. Assessment of 11-β hydroxysteroid dehydrogenase (11-βHSD1) 4478T> G and tumor necrosis factor-α (TNF-α)-308G> A polymorphisms with obesity and insulin resistance in Asian Indians in North India. Mol Biol Rep 2013;40(11):6261-6270
52. Fernandez-Veledo S, Vila-Bedmar R, Nieto-Vazquez I, Lorenzo M. c-Jun N-terminal kinase 1/2 activation by tumor necrosis factor-α induces insulin resistance in human visceral but not subcutaneous adipocytes: reversal by liver X receptor agonists. J Clin Endocrinol Metab 2009;94(9):3583-3593
53. Han D, Hanawa N, Saberi B, Kaplowitz N. Mechanisms of liver injury. III. Role of glutathione redox status in liver injury. Am. J. Physiol. Gastrointest. Liver Physiol 2006;291(1):G1-G7
54. Han D, Hanawa N, Saberi B, Kaplowitz N. Hydrogen peroxide and redox modulation sensitize primary mouse hepatocytes to TNF-induced apoptosis. Free Radic Biol Med 2006;41(4):627-639
55. Dou L, Zhao T, Wang L, Huang X, Jiao J, Gao D, Zhang H, Shen T, Man Y, Wang S, Li J. miR-200s contribute to interleukin-6 (IL-6)-induced insulin resistance in hepatocytes. J Biol Chem 2013;288(31):22596-22606
56. Castañeda S, Remuzgo-Martínez S, López-Mejías R, Genre F, Calvo-Alén J, Llorente I, Aurrecoechea E, Ortiz AM, Triguero A, Blanco R, Llorca J. Rapid beneficial effect of the IL-6 receptor blockade on insulin resistance and insulin sensitivity in non-diabetic patients

with rheumatoid arthritis. Clin Exp Rheumatol 2019;37(3):465473.
57. Akbari M, Hassan-Zadeh V. IL-6 signalling pathways and the development of type 2 diabetes. Inflammopharmacology 2018;26(3):685-698.
58. Ji C, Chen X, Gao C, Jiao L, Wang J, Xu G, Fu H, Guo X, Zhao Y. IL-6 induces lipolysis and mitochondrial dysfunction but does not affect insulin-mediated glucose transport in 3T3-L1 adipocytes. J Bioenerg Biomembr 2011;43(4):367.