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## Anti-Diabetic and Anti- Fibrotic effects of Gold and Silver Nano-particles on Diabetic Nephropathy induced experimentally

Amal Hamza<sup>1,2</sup>, Heba Bashuaib<sup>1</sup>

<sup>1</sup> Biochemistry Department, Faculty of Science – Alfaisalia, King Abdulaziz University, **Kingdom of Saudi Arabia**

<sup>2</sup> Biochemistry and Nutrition Department, Faculty of Women, Ain Shmas University, **Egypt**

\*Correspondence: [ahamza@kau.edu.sa](mailto:ahamza@kau.edu.sa) Accepted: 06 Jan. 2018 Published online: 04 Mar. 2018

The application of nanoparticles in the clinical field is of great interest. The aim of this study is to explore the hypothesis that gold and silver nanoparticles could play a positive role in ameliorating the biochemical changes accompanied diabetic nephropathy in experimental model. Rats were treated orally with silver or gold nanoparticles for 21 days after DN induction. Our results showed significant regression of blood glucose level and serum AGEs accompanied by increase in serum insulin level in DN treated with silver, and gold nanoparticles compared to DN induced group. Also, gold and silver nanoparticles remarkably recovered kidney function biomarkers including urea, creatinine, uric acid albumin and micro albumin in treated group as compared to DN induced group. In addition, kidney fibrotic markers cystatine C, fibronectin, TGF- $\beta$ , Laminine and transferine, showed progressive enhancement in DN treated with silver and gold nanoparticles compared to DN induced experimentally. In Conclusion; the present study highlighted the biological effect of AgNPS, and AuNPS in ameliorating DN induced experimentally through anti-fibrotic and anti-diabetic effect.

**Keywords:** Diabetic nephropathy, Gold nanoparticles, Silver nanoparticles, fibrotic markers, kidney functions, diabetic biomarkers.

### INTRODUCTION

Diabetes mellitus is a reason for chronic kidney dysfunction worldwide (Parchwani and Upadhyah, 2012). Diabetic nephropathy is a clinical disorder described by albuminuria (>300 mg/day or >200 mcg/min) affirmed on no less than two events 3-6 months separated, permanent and irreversible abatement in glomerular filtration rate (GFR) and blood vessel hypertension (Adler et al., 2003).

Diabetic nephropathy happens because of an interaction amongst hemodynamic and metabolic factors. Hemodynamic components that add to the advancement of diabetic nephropathy incorporate expanded systemic and intra glomerular pressure, and in addition actuation of

vasoactive hormone pathways including the rennin angiotensin system and endothelin. Nuclear transcription factors, for example, nuclear factor kappa- light-chain-enhancer of activated B cells (NF-kB) and different growth factors and glucose pathways are likewise initiated inside the diabetic kidney and result in enhanced oxidative stress, renal polyol arrangement, and the accumulation of advanced glycation end products (AGEs) (Arya et al., 2010). In the underlying phases of diabetic nephropathy, expanded kidney size and changed doppler indicators might be the early morphological indications of renal dysfunction, while proteinuria and GFR are the best markers of the level of the harm (Vujicic et al., 2012).

Nobel metal nanoparticles are used for different biomedical applications and noteworthy outcomes have been acquired (Rajathi et al., 2012a) particularly, gold nanoparticles (AuNPs) are utilized for the treatment of different illnesses (Dhas et al., 2012), because of their unique role optical, chemical and biological properties (Kumar et al., 2011). The therapeutic effect of gold nanoparticles (AuNPs) depends on their particular physical properties and, their capacity to cooperate with tumors and damage cancer cells. Silver nanoparticles are of intrigue due to the unique properties (e.g., size and shape depending optical, electrical, and magnetic properties) which can be applied into antimicrobial applications, biosensor materials, composite filaments, cryogenic superconducting materials, cosmetic products, and electronic components (Korbekandi and Iravani, 2012).

Silver nanoparticles (AgNPs) are today a standout amongst the most normally utilized nanomaterials both in regular daily life, and in inquire about laboratory research, silver nanoparticles are likewise ordinarily utilized as a part of therapeutic practice as a basic piece of both surgical and nonsurgical equipment (Pantic, 2014).

Based on the natural impact of gold and silver nanoparticles we hypothesis their conceivable impact in improving DN actuated experimentally through its possible hypoglycemic and anti-fibrotic effect.

## MATERIALS AND METHODS

### Chemicals

Streptozotocin (STZ) was supplied from Sigma-Aldrich Chemicals Company St. Louis, USA. Gold nanoparticles (AuNPs), and Silver nanoparticles (AgNPs) were purchased from Nanotech Egypt for Photo Electronics. kits used for the quantitative determination of different parameters were purchased from HUMAN co., Germany and NOVA. Beijing, China.

### Experimental Design

Sixty male adult albino rats weighing 220-230 g were used in this study. Rats were supplied from the Animal House Colony of King Fahd Medical Research Centre, Jeddah. After the acclimatization period. Rats were controlled with a 12 h light/dark cycle. Animals were kept in special cages at 20–22 °C and humidity (60%) at King Fahd Medical Research Centre Animal Facility Breeding Colony. Standard diet according to

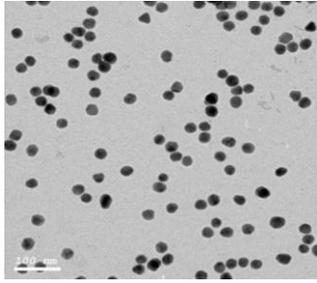
(A.O.A.C., 1995) and water were freely supplied. Biological experiments were approved by the Ethical Committee of King Fahd Medical Research Centre, Jeddah, and KSA. Rats were divided into two groups 30 rats each. Group (A) healthy control group injected with 1 ml saline IP daily. Group (B): DN induced group by single IP injection of STZ (60 mg/kg BW). Rats were considered diabetic when glucose level reach 250mg/dl or higher. After 15 days group A, B were further divided into 3 groups each. Healthy control group was further treated either with gold or silver nano-particles in a dose of 0.25 mg/Kg bw IP daily for 21 days according to Karithick et al.,(2014) and Al-Kaldi et al., (2014) respectively. Group B was further divided into three groups DN positive control group, DN treated with either gold or silver nanoparticles in the same dose. At the end of the experimental period, rats were fasted overnight and subjected to diethyl ether anesthesia. The blood samples were immediately withdrawn from the retro-orbital venous plexus for biochemical analysis. Then rats were sacrificed by cervical dislocation, kidney and pancreas were separated, cleaned and dried, then it was preserved in formalin saline 10 % for histopathological examinations.

### Preparation and characterization of Gold and Silver nanoparticles:

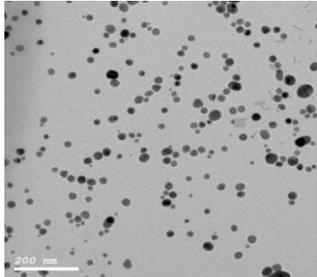
Gold and Silver nanoparticles were prepared by chemical reduction method as reported by Turkevich (1951) and Pal et al., (2009) respectively. UV absorption spectra were obtained on an Ocean Optics USB2000+VIS-NIR Fiber optics spectrophotometer. *Transmission electron microscopy* (TEM) was performed on JEOLJEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV, respectively, Fig (2) and (3) respectively.

**Table (A), Properties of Gold & Silver Nanoparticles**

Properties	Gold Nanoparticles	Silver Nanoparticles
Appearance (Color)	Pink	Yellow
Concentration	198 micro gm/ml	500 ppm (0.5mg/ml)
Solubility	198 micro gm/ml	Water, Ethanol
Optical Prop. (Abs.)	$\lambda_{\max} = 522$ nm	$\lambda_{\max} = 408$ nm
Avg. Size(TEM)	$13 \pm 1$ nm	20 nm



**Figure (A) High resolution transmission electron image (AgNPs)**



**Figure (B) High resolution transmission electron image of (AuNPs)**

#### Laboratory Measurements:

Blood glucose were detected by Peripheral blood from the tail vein by (one touch sure step meter life scan, CA). Creatinine, urea, uric acid, total protein and albumin were measured using kits purchased from HUMAN CO., Germany. Serum insulin, micro albumin, fibronectine, laminine, advanced glycation end products, transforming growth hormone beta, transferrine and cystatine C were measured using kits use a double-antibody Sandwich enzyme- linked immunoabsorbent assay according to manufacture instructions of NOVA Co. assay kit Beijing, China.

#### Histopathological examination for Kidney and Pancreases

Dissection samples were taken from Kidney and Pancreases of rats in various groups and settled in 10% formal saline for 24 hours. Washing was finished with tap water then serial dilutions of liquor (methyl, ethyl and absolute ethyl) were utilized for decrease hydration. Samples were cleared in xylene and installed in paraffin at 56 degree in hot air stove for 24 hours. Paraffin bees wax tissue blocks were set up for separating at 4 microns thickness by sledge microtome. The acquired tissue areas were gathered on glass slides, deparaffinized and stained by hematoxylin and eosin stain and analyzed through the electric

light magnifying lens as per (Banchroft et al., 1996).

#### Statistical analysis

Information was factually examined by comparing the values for various test groups with the estimations of individual ordinary ones. Results were communicated as mean  $\pm$  SE. significant change among groups were broke down utilizing analysis of variance (ONE-WAY ANOVA) combined with post-Hoc least significant difference (LSD). ANOVA at  $\leq 0.05$  was viewed as significant.

#### RESULTS

Results presented in table (1) showed significant elevation in blood glucose, serum and urinary urea, creatinine and nitrogen level in DN induced group comparing to healthy group which confirm DN induction ( $P \leq 0.05$ ).

Table (2) illustrates blood level of glucose, serum AGE, insulin and insulin resistance. It is clear from the results a significant elevation of blood glucose and AGE level in DN group as compared to healthy group ( $P \leq 0.05$ ). Treatment with either gold or silver nanoparticles showed significant regression in these biomarkers. On the other hand, insulin level significantly increased in gold and silver nanoparticles treated group.

Results in table (3-4) revealed significant elevation in serum and urinary urea, creatinine and uric acid level in DN group comparing to healthy control group, while gold and silver nanoparticles treated group showed significant regression in these markers ( $P \leq 0.05$ ). Also, creatinine clearance significantly decreased by gold and silver nanoparticles treatment.

Table (5) showed the concentration of serum albumin and total protein as well as urinary albumin and urinary microalbumin in all experimental groups. It could be seen from this table that DN group showed significant regression in serum albumin and total protein level while urinary albumin and microalbumin were significantly elevated as compared to healthy groups. Groups treated with either gold or silver nanoparticles showed significant amelioration of these biomarkers ( $P \leq 0.05$ ).

Regarding the results of TGF- $\beta$ , transferring and cystatine C. It is clear that there was significant elevation in DN induced group comparing to healthy controls ( $P \leq 0.05$ ). On the other hand, DN induced group treated with gold or silver nanoparticles showed significant regression in the investigated biomarkers (Table 6).

**TABLE (1) Level of blood glucose, creatinine, urea, and nitrogen level in serum and urine in all healthy and diabetic groups (15 days) (Mean  $\pm$  SE)**

Groups	Healthy Control Group (A)	Diabetic Nephropathy Group (B)
Blood glucose (mg/dl)	79.44 $\pm$ 1.79	415.22 $\pm$ 22.2
Serum Creatinine (mg/dl)	0.67 $\pm$ 0.02	1.48 $\pm$ 0.073 <sup>a</sup>
Urinary Creatinine (mg/dl)	74.92 $\pm$ 1.93	95.25 $\pm$ 1.90 <sup>a</sup>
Serum Urea (mg/dl)	69.07 $\pm$ 1.07	111.00 $\pm$ 3.16 <sup>a</sup>
Urinary Urea(g/l)	63.28 $\pm$ 1.72	82.90 $\pm$ 1.56 <sup>a</sup>
Serum nitrogen(mg/dl)	32.46 $\pm$ 2.15	52.17 $\pm$ 1.48 <sup>a</sup>
Urinary nitrogen (g/l)	29.74 $\pm$ 0.80	38.96 $\pm$ 0.73 <sup>a</sup>

(a)Significant changes as compared to Healthy group ( $P \leq 0.05$ )**TABLE (2): Concentration of Blood glucose level, serum Advanced glycation end products and Insulin in all experimental groups (Mean  $\pm$ SE).**

Groups	Blood glucose mg/dl	Advanced glycation end Product (ng/ml)	Insulin mU/L
Healthy Control	104.90 $\pm$ 7.26	86.42 $\pm$ 1.47	2.20 $\pm$ 0.07
Healthy + AuNPS	121.50 $\pm$ 9.84 <sup>d</sup>	85.96 $\pm$ 1.11 <sup>d</sup>	2.17 $\pm$ 0.07 <sup>d</sup>
Healthy + AgNPS	95.70 $\pm$ 2.77 <sup>d</sup>	85.95 $\pm$ 2.05 <sup>d</sup>	2.10 $\pm$ 0.10 <sup>d</sup>
DN Group	348.80 $\pm$ 55.98 <sup>a,b,c</sup>	114.68 $\pm$ 1.83 <sup>a,b,c</sup>	1.15 $\pm$ 0.01 <sup>a,b,c</sup>
DN + AuNPS	176.10 $\pm$ 19.86 <sup>f</sup>	94.71 $\pm$ 1.00 <sup>d</sup>	1.72 $\pm$ 0.03 <sup>d</sup>
DN + AgNPS	331.10 $\pm$ 56.07	100.82 $\pm$ 2.11 <sup>d</sup>	1.59 $\pm$ 0.05 <sup>d</sup>

(a)Significant changes as compared to Healthy group. (b) Significant changes as compared to healthy AuNPs, (c) significant changes as compared to healthy AgNPs, (d) significant changes as compared to diabetic nephropathy induced group. ( $P \leq 0.05$ ). DN: diabetic nephropathy, AuNPS: gold nanoparticules, AgNPS: silver nanoparticles**TABLE (3): Concentration of Urea and creatinine in Serum of all experimental groups (Mean  $\pm$ SE).**

Parameters	Urea mg/dl	Creatinine mg/dl
Healthy Control	80.10 $\pm$ 1.86	0.75 $\pm$ 0.03
Healthy + AuNPs	82.60 $\pm$ 1.73 <sup>d</sup>	0.84 $\pm$ 0.04 <sup>d</sup>
Healthy + AgNPs	81.88 $\pm$ 2.35 <sup>d</sup>	0.71 $\pm$ 0.03 <sup>d</sup>
DN Group	162.25 $\pm$ 4.84 <sup>a,b,c</sup>	2.36 $\pm$ 0.12 <sup>a,b,c</sup>
DN + AuNPs	102.66 $\pm$ 5.51 <sup>d</sup>	2.01 $\pm$ 0.12
DN + AgNPs	112.16 $\pm$ 7.37 <sup>d</sup>	2.07 $\pm$ 0.06 <sup>d</sup>

(a)Significant changes as compared to Healthy group. (b)significant changes as compared to healthy AuNPs, (c)significant changes as compared to healthy AgNPs, (d)significant changes as compared to diabetic nephropathy induced group. ( $P \leq 0.05$ ). DN: diabetic nephropathy, AuNPS: gold nanoparticules, AgNPS: silver nanoparticles.

**TABLE (4): Concentration of urinary urea, creatinine and creatinine clearance in all experimental groups (Mean  $\pm$  SD).**

Groups	Urea g/l	Creatinine mg/24h	Creatinine Clearance (ml/min)
Healthy Control	66.01 $\pm$ .29	85.00 $\pm$ 3.22	1.17 $\pm$ 0.44
Healthy + AuNPS	68.02 $\pm$ .60 <sup>d</sup>	82.09 $\pm$ 3.06 <sup>d</sup>	0.58 $\pm$ 0.29
Healthy + AgNPS	65.87 $\pm$ .70 <sup>d</sup>	79.49 $\pm$ .93 <sup>d</sup>	0.54 $\pm$ 0.18
DN groups	82.91 $\pm$ .234 <sup>a,b,c</sup>	134.86 $\pm$ 6.42 <sup>a,b,c</sup>	0.83 $\pm$ 0.41 <sup>a</sup>
DN + AuNPS	69.40 $\pm$ 1.36 <sup>d</sup>	90.94 $\pm$ 3.66 <sup>d</sup>	0.76 $\pm$ 0.52
DN + AgNPS	75.23 $\pm$ .97 <sup>d</sup>	93.77 $\pm$ 1.13 <sup>d</sup>	0.54 $\pm$ 0.30

(a)Significant changes as compared to Healthy group. (b)significant changes as compared to healthy AuNPs, (c)significant changes as compared to healthy AgNPs, (d)significant changes as compared to diabetic nephropathy induced group. (P $\leq$ 0.05). DN: diabetic nephropathy, AuNPS: gold nanoparticules, AgNPS: silver nanoparticles

**TABLE (5): Concentration of serum and urinary Albumin, Total protein in serum and urinary microalbumin of all experimental groups (Mean  $\pm$  SE).**

Parameters Groups	Albumin(g/dl)	Total protein(g/dl)	Urinary Albumin (g/dl)	Microalbumin (ug/ml)
Healthy Control	3.52 $\pm$ 0.15 <sup>b,d</sup>	7.71 $\pm$ 0.67	1.57 $\pm$ 0.02	5.01 $\pm$ 0.16
Healthy + AuNPS	3.39 $\pm$ 0.21 <sup>d</sup>	7.64 $\pm$ 0.60 <sup>d</sup>	1.60 $\pm$ 0.03 <sup>d</sup>	5.35 $\pm$ 0.13 <sup>d</sup>
Healthy + AgNPS	3.56 $\pm$ 0.24 <sup>d</sup>	7.80 $\pm$ 0.72 <sup>d</sup>	1.54 $\pm$ 0.05 <sup>d</sup>	5.37 $\pm$ 0.12 <sup>d</sup>
Diabetic Nephropathy	2.69 $\pm$ 0.38 <sup>a,b,c</sup>	4.22 $\pm$ 0.51 <sup>a,b,c</sup>	2.54 $\pm$ 0.08 <sup>a,b,c</sup>	8.18 $\pm$ 0.23 <sup>a,b,c</sup>
DN + AuNPS	3.02 $\pm$ .42 <sup>b</sup>	5.26 $\pm$ 0.22	1.75 $\pm$ 0.03 <sup>d</sup>	6.79 $\pm$ 0.16 <sup>d</sup>
DN + AgNPS	287 $\pm$ 0.37	5.44 $\pm$ 0.34	1.83 $\pm$ 0.03 <sup>d</sup>	7.32 $\pm$ 0.20 <sup>d</sup>

(a)Significant changes as compared to Healthy group. (b) Significant changes as compared to healthy AuNPs, (c) significant changes as compared to healthy AgNPs, (d) significant changes as compared to diabetic nephropathy induced group. (P $\leq$ 0.05). DN: diabetic nephropathy, AuNPS: gold nanoparticules, AgNPS: silver nanoparticles

**TABLE (6) Concentration of Serum Transforming growth factor (TGF- $\beta$ ), cystatine C and Urinary Transferrine of all experimental groups (Mean  $\pm$  SE)**

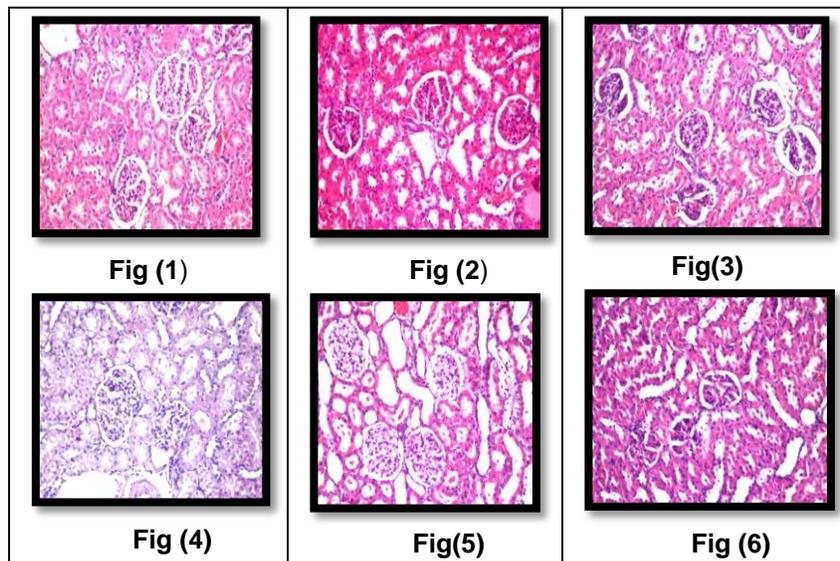
Parameters	Transforming Growth factor (pg/ml)	Transferrine (pg/ml)	Cystatine C (ng/ml)
Healthy Control	50.74 $\pm$ 0.75	908.30 $\pm$ 11.9	99.24 $\pm$ 1.66
Healthy + AuNPS	54.26 $\pm$ 0.77 <sup>d</sup>	906.10 $\pm$ 3.14 <sup>d</sup>	104.67 $\pm$ 1.41 <sup>d</sup>
Healthy + AgNPS	54.49 $\pm$ 0.95 <sup>d</sup>	908.60 $\pm$ 2.99 <sup>d</sup>	103.84 $\pm$ 1.41 <sup>d</sup>
Diabetic	118.73 $\pm$ 1.66 <sup>a,b,c</sup>	1104.25 $\pm$ 1.23 <sup>a,b,c</sup>	145.42 $\pm$ 0.87 <sup>a,b,c</sup>
DN + AuNPS	91.63 $\pm$ 1.86 <sup>d</sup>	1034.61 $\pm$ 3.02 <sup>d,f</sup>	119.93 $\pm$ 1.48 <sup>d</sup>
DN + AgNPS	108.10 $\pm$ 1.78 <sup>d</sup>	1087.13 $\pm$ 1.66 <sup>e</sup>	134.16 $\pm$ 0.77 <sup>d</sup>

(a)Significant changes as compared to Healthy group. (b) Significant changes as compared to healthy AuNPs, (c) significant changes as compared to healthy AgNPs, (d) significant changes as compared to diabetic nephropathy induced group (P $\leq$ 0.05). DN: diabetic nephropathy, AuNPS: gold nanoparticules, AgNPS: silver nanoparticles.

**TABLE (7): Concentration of Fibronectin, and Laminine in Serum of all experimental groups (Mean  $\pm$  SE)**

Groups	Fibronectin (ng/ml)	Laminin(pg/ml)
Healthy Control	5.79 $\pm$ 0.16	194.85 $\pm$ 0.91
Healthy + AuNPS	6.01 $\pm$ 0.29 <sup>d</sup>	204.87 $\pm$ 8.34 <sup>d</sup>
Healthy + AgNPS	5.93 $\pm$ 0.23 <sup>d</sup>	200.42 $\pm$ 2.54 <sup>d</sup>
Diabetic Nephropathy	9.18 $\pm$ 0.22 <sup>a,b,c</sup>	274.18 $\pm$ 0.98 <sup>a,b</sup>
DN + AuNPS	7.04 $\pm$ 0.30 <sup>d,f</sup>	243.75 $\pm$ 1.91 <sup>d,f</sup>
DN + AgNPS	8.52 $\pm$ 0.27 <sup>e</sup>	267.07 $\pm$ 2.02 <sup>e</sup>

(a) Significant changes as compared to Healthy group. (b) Significant changes as compared to healthy AuNPs, (c) significant changes as compared to healthy AgNPs, (d) significant changes as compared to diabetic nephropathy induced group ( $P \leq 0.05$ ). DN: diabetic nephropathy, AuNPS: gold nanoparticles, AgNPS: silver nanoparticles.



**Figure (1)** A photograph of kidney rat in healthy control group showing normal histological structure of the glomeruli tubules or the cortex H&E x40

**Figure (2)** a photograph of kidney of rats in healthy (AgNPs) showing normal histological structure H&E x 40

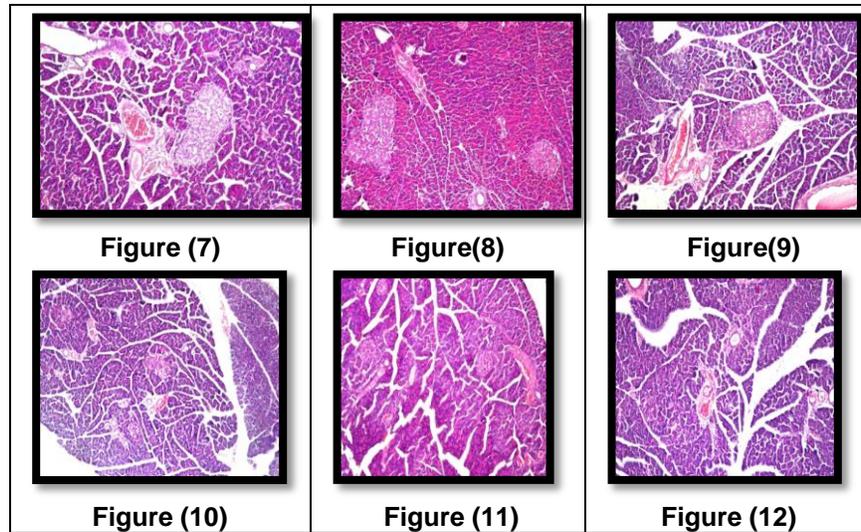
**Figure (3)** A photograph of kidney rat in healthy (AuNPs) showing normal histological structure H&Ex40

**Figure (4)** A photograph of kidney of rat in DN group showing degeneration in the tubular lining epithelium with swelling and vacuolization of the endothelium cells

**Figure(5)** A photograph of kidney of DN induced rats treated with AgNPs showing vacuolization of endothelial cells lining the tufts with cystic dilatation in some cortical tubules H&E x40 lining the glomerulas tufts H&E x40

**Figure (6)** A photograph of rat kidney in DN induced group treated with AuNPs showing intact histological structure of the glomeruli and tubules at the cortex H&E x40.

## Pancreatic Sections:



**Figure (7)** A photograph of pancreas in healthy control group showing normal histological structure of the islets of Langerhans cells H&Ex40.

**Figure (8)** A photograph of pancreas of rats in healthy (AgNPs) showing normal histological structure H&E x16.

**Figure (9)** A photograph of pancreas of rats in healthy (AuNPs) showing normal histological structure H&Ex40.

**Figure (10)** A photograph of pancreas of rats in DN induced rat showing atrophy with small size islands of Langerhans cells in diffuse manner H&E x 16.

**Figure (11)** A photograph of pancreas of DN induced rats treated with AgNPs showing atrophy with small size islands of Langerhans in diffuse manner H&Ex16

**Figure (12)** A photograph of pancreas of rat in DN induced group treated with AuNPs showing atrophy and absence of islands of Langerhans cells in diffuse manner H&Ex16.

Concentration of serum Laminin and Fibronectin are illustrated in table (7). Significant elevation in fibronectin and laminin were observed in DN group accompanied by significant regression when treated with gold and silver nanoparticles ( $P \leq 0.05$ ).

Our results showed that gold nanoparticles have stronger ameliorative effect on DN complications than silver nanoparticles.

Results of histopathological examination of kidney and pancreatic tissues are illustrated through figures from (1-12).

## DISCUSSION

High blood glucose is known to be an

important reason for DN. The control of glucose can keep the event and advancement of DN. In particular, the control of blood glucose is one of the vital measures utilized for the counteractive action and treatment of DN (Xu et al., 2012). The results of the present investigation demonstrated noteworthy elevation in blood glucose and AGEs level in DN initiated group accompanied by significant decline in insulin level when contrasted with healthy control. This is expected due to streptozotocin (STZ) which can specifically damage insulin-producing pancreatic endocrine cells and initiate experimental hyperglycemia (Wang et al., 2014).

STZ is generally utilized as an investigational medicate for diabetes inquire because of its

particular toxicity associated with pancreatic  $\beta$ -cells (Akbarzadeh et al., 2007). Low affinity glucose transporter-GLUT 2 of  $\beta$ -cells transports STZ into the cell and causes alkylation of DNA and irreversible necrosis of  $\beta$  cells (Goud et al., 2015).

This event formed by polyol pathway and increased extracellular matrix (ECM) synthesis activated by high level of glucose transporter 1 (GLUT 1) in renal mesangial cells, high blood glucose levels may cause glycation of several structural and functional proteins including plasma protein (Khan et al., 2009). The elevated level of glucose starts forming covalent adducts with plasma protein through a non-enzymatic pathway known as glycation, protein glycation reaction leading to AGEs are expected to be the major causes of different diabetic complications (Negre-Salvayre et al., 2009). These results are supported by Singh et al., (2014).

It is clear from our results that IP treatment of DN induced group by either gold or silver nanoparticles showed significant regression in blood glucose and serum AGEs concentration as compared to DN induced group, while insulin level increased by silver and gold nano particles treatment. Gold nanoparticles affect blood glucose level by exerted insulin as an effect on peripheral tissues by either promoting the absorption of glucose, preventing hepatic gluconeogenesis, absorption of glucose into the muscle and adipose tissues or through the catalysis of regeneration process and revitalization of the remaining  $\beta$ -cells (Al-Azzawie and Yaaqoob, 2016).

The hypoglycemic effect of silver nanoparticles may be through its effect in improving glucose utilization and metabolism through its potent influence on enhancement of hepatic glycogenesis through actions on insulin signaling pathway (Jansen et al., 2009). Our results are in agreement with Alkaladi et al (2014). AuNPs affect the serum insulin level by increasing the pancreatic secretion of insulin from  $\beta$  – cells of islets of langerhans, these results are in agreement with Dhas et al (2016). Also. AuNPs could bring up insulin level in the plasma to a normal level which slowly contributed to glucose metabolism this result was explained by Sengani and Rajeswari (2017).

On the other hand, AgNPs could decrease AGEs level through its effective power on glucose status and reducing the level of GLUT 1 in renal mesangial cells which inhibit the production of AGEs (Singh et al., 2014). This result in agreement with Sengani and Rajeswari (2017).

In the present investigation DN initiated group demonstrated an elevation in Serum urea, creatinine, and uric acid indicated noteworthy rise in DN actuated group contrasted with healthy control group. An increase in urea, creatinine, and uric acid level is seen when kidney isn't working legitimately. Augmentation of blood urea, serum creatinine, and uric acid level in addition of blood glucose level obviously shows that the elevated blood glucose level induce kidney dysfunction, so kidney isn't working appropriately these outcomes affirm the enlistment of DN in our examination.

Additionally, Anjaneyulu et al., (2004) has discovered that increased urea and serum creatinine in diabetic rats demonstrates dynamic renal damage. Likewise it was discovered comparable outcome that, creatinine levels were essentially higher in the diabetic group compared by the control group (Güngör et al., 2006). Diabetes mellitus causes micro and macro vascular changes in the body and this prompts diabetic nephropathy. Hyperglycemia will prompt hyper filtration and subsequently expanded glomerular filtration rate (Kamal, 2014).

DN prompted group treated with gold or silver nanoparticles demonstrated a lessened level in serum urea, creatinine, and uric acid because of controlling impact of gold nanoparticles on glucose level through insulin empowering impact on pancreatic cells (Karthick et al., 2014). AuNPs demonstrated an extensive impact on these markers close to normal level these outcomes are as per Sangani and Rajeswari (2016). Likewise, AuNPs seemed to diminished level of serum urea, creatinine, and uric acid viably and could be clarified by the regenerative capacity of the renal tubules this outcomes upheld by Daisy and Saipriya, (2012). AgNPs has successful part on blood glucose level which prompts lessening of hyperfiltration rate that manage the renal capacities (Kamal, 2014).

With respect to fibronectin level, our investigation demonstrated critical increase in DN prompted group. Elevation of serum fibronectin in DN induced group relates to changing in concentration level of fibronectin in tissues and body fluids caused by diabetes mellitus which was credited to increase synthesis caused by proinflammatory cytokins, and release of extracellular matrix and cell surface fibronectin into circulating plasma (Kanters et al., 2001). Likewise hyperglycemia or high glucose level outcomes in an augmented expression of gene responsible for fibronectin synthesis (Wyczalkowska-Tomasik et al., 2012). We

discovered noteworthy relapse in fibronectin level by AuNPs and AgNPs treatment. AuNPs controlling fibronectin level through its impact on diminishing AGEs. AGE-aggregation improved arrangement of intra/extracellular proteins, and smothered protein corruption this outcomes runs hands to hands with Wyczalkowska-Tomasik et al., (2012). The role of AgNPs in hindrance fibronectin formation because of its impact on the function of proteins, and DNA replication this outcomes supported by Pantic, (2014).

Our study expressed an elevation of serum TGF- $\beta$ 1 in DN actuated group in contrast with control group. Hyperglycemia is the standard factor for the metabolic and structural modifications in diabetes; the pathways incorporate generation of TGF- $\beta$ 1 (Kanwar et al., 2011). The high blood glucose level increase the level of mRNA for TGF- $\beta$ 1 in the kidney. Blockade of TGF- $\beta$  impacts prevents diabetic renal hypertrophy in trial models of diabetic nephropathy (Lane et al., 2001). Expanded expression of TGF- $\beta$ 1 protein in diabetic glomerulosclerosis was seen in podocyte of diabetic animals (Baba et al., 2005). Furthermore, TGF- $\beta$ 1 suppresses matrix degrading enzymes by inhibition of the MMPs synthesis and enhance production of TIMPs (Wyczalkowska-Tomasik et al., 2012).

Controlling blood glucose level through the capacity of gold nanoparticles prompts controlled renin angiotensin system (RAAS) blocker are related with a concealment role of TGF- $\beta$ 1, our outcomes were upheld by Tomasik et al., (2012). Besides, through the part of AuNPs in controlling blood glucose together with glycated albumin and advanced glycation end products AGEs restrain TGF- $\beta$ 1 in mesangial cells *in vitro*. These outcomes are in concurrences with Leeson (2013). High glucose and the glycated albumin and AGE actuate TGF- $\beta$  over expression in mesangial cells in culture (Lee et al., 2004), not in podocytes (Iglesias-de la Cruz et al., 2002). In human and experimental diabetic nephropathy, some mesangial cells demonstrate immunoreactivity for TGF- $\beta$ 1 Enhanced expression of glomerular TGF- $\beta$ 1 is likewise watched fundamentally in podocytes of diabetic animals (Baba et al., 2005, Herbach et al., 2009). This enhancement is identified with the role of metal like silver nanoparticles in glucose metabolism and the relationship of their insufficiency with diabetes. Likewise., Alkaladi et al., (2014) reported that AgNPs has a role in glucose support which has leading to control

serum TGF- $\beta$ 1.

Several examinations demonstrate that a lessening in serum albumin, even inside the range that is at present thought about ordinary, shows higher renal hazard (Babazono et al., 2009). In the present investigation DN induced group demonstrated a decline in concentration level of serum total protein when contrasted with control group, this could be because of diminished amino acid take-up, significantly diminished concentration of a several fundamental amino acids take-up, an increase in change rate of glycogenic amino acids to CO<sub>2</sub> and H<sub>2</sub>O, and a lessening in protein synthesis secondary to a diminished amount and availability of mRNA (Ahmed, 2005).

DN induced group treated with gold nanoparticles demonstrated a significant increase in total protein when contrasted with DN prompted group, the inflamed cells with free sulfur, nitrogen and oxygen have greater proclivity towards gold atoms (Tiwari et al., 2011). Various investigations have exhibited that nanoparticles interaction with serum proteins and cell membrane receptors were controlled by the nanoparticles configuration (size, shape and charge), in an impact of affecting cell take-up, gene expression, and toxicity (Gatoo et al., 2014). Likewise, serum albumin demonstrated a critical increment in DN initiated group tread with AuNPs when contrasted with the DN instigated group. Findings of the accompanying biological markers were supported by Sengani et al., (2016).

Serum total protein and albumin demonstrated a critical elevation in DN induced group treated with AgNPs when contrasted with DN actuated group this identified with the role of AgNPs in improvement the insulin secretion by  $\beta$ -cells which prompts glucose metabolism which is one of the pathway for protein catabolism. Likewise, these outcomes might be clarified by the role of AgNPs in restraint the function of proteins and DNA replications (Pantic, 2014).

Microalbuminuria is a sensitive and early marker of diabetic nephropathy, different types of renal damage, and endothelial permeability all through the vascular tree. It predicts progression of kidney disease prompting end stage renal disease (ESRD) (Drummond and Mauer, 2002, Konta et al., 2007). Microalbumin has been considered as the most punctual marker of DN. Which is a significant extent of people with diabetes could have renal dysfunction earlier or even without passing the stage of micro albuminuria, the highest quality level for early diagnosis (Rigalleau et al., 2007).

We found a significant rise in urinary albumin, and microalbumin in DN induced group when contrasted with control group. This elevation in these biomarkers characterized by hyperglycemia coming about because of deformities in insulin secretion, insulin activity, or both which is prompting different complications including diabetic nephropathy (King et al., 2005). These outcomes in concurrences with Rahman et al., (2012) who found that urinary albumin excretion rate are elevated in diabetes mellitus. On the other hand, there was critical regression in urinary albumin and microalbumin of DN actuated group treated with either AgNPs or AuNPs.

Silver nanoparticles can influence the level of urinary albumin and microalbumin through their role in elevating serum insulin and lessening the blood glucose level through improvement of hepatic glycogenesis (Alkaladi et al., 2014).

Our investigation illustrated that serum laminin in DN prompted group was fundamentally increased when contrasted with control group. Laminin has a role in the support of structural integrity and particular permeability capacity of the glomerular basement membrane this could be defected through increase of serum laminin. Investigation of Ha et al., (1999) is in concurrences with our outcomes that irregularities in laminin deposition have been accounted for in several renal illnesses, including diabetic nephropathy. Likewise, the combination of expanded renal laminin immunoreactivity and reduction its mRNA levels in type II diabetes proposes diminished turnover of laminin as a potential mechanism of laminin accumulation by matrix metalloproteinases (MMP) and protection of laminin to breakdown.

Noteworthy enhancement change was seen by AuNPs and AgNPs treatment. This identified with the role of AuNPs in elevating the serum insulin level which thus have an regulatory role in renal extracellular matrix (ECM) synthesis because of renal cortical insulin receptor enhancement (Mariappan et al., 2007). While AgNPs have an important role in increase level of insulin which has an regulatory part in serum laminin this outcome upheld Mariappan et al., (2007). The investigation of Mariappan et al., (2007) found that high insulin may invigorate combination of small amount of laminin- $\beta$ 1, which overtime contribute to critical development of expansion of matrix in the kidney in type 2 diabetes, and give extra reasonable to keeping up tight control of plasma glucose in counteracting renal complexities in diabetes.

The present investigation demonstrated that serum cystatine c was significantly elevated in DN prompted group when contrasted with control group. The minimal renal damage caused by the rise in blood glucose which will bring about a noteworthy rise level in serum cystatine c before the presence of CKD markers like this outcomes are supported by Borges et al., (2010).

It was reported by Surendar et al., (2009) that cystatine c level were most elevated in type 2 diabetes patients without micro albuminuria, which recommend that measurement of cystatine c in serum can be utilized for predicting beginning of nephropathy in type 2 diabetic patients with norm albuminuria [early nephropathy].

The present study demonstrated that DN treated with gold and silver nanoparticles demonstrated a noteworthy enhancement in serum cystatine c when contrasted with DN prompted group. This could be clarified by the role of AuNPs on the level of proteins content and AgNPs impact on serum cystatine c because of it is anti-inflammatory impact (Daisy and Saipriya, 2012).

In the present examination urinary transferrin was essentially elevated in DN instigated group when contrasted with control group, this identified with affectability of transfer to glomerular damage in diabetic patients. Urinary transferrin excretion demonstrates a good association with the improvement of micro albuminuria in type 2 diabetes. This outcome is in concurrence with Cheng, (2013).

Silver nanoparticles treated DN incited group demonstrated a noteworthy improvement in transferrine level when contrasted with DN prompted group this diminishment happens because of an increase in the aggregate entropy of the proteins on the nanoparticles surface and nanospesfic interaction between the nanoparticles surface and proteins, cell take-up of AgNPs is observed to be adjusted by their interaction with proteins such as transferrin (Monteiro-Riviere et al., 2013).

Regarding the histopathological studies on kidney and pancreatic tissue, It is greatly support the biochemical results. Kidney section of DN induced group showed degeneration in the tubular lining epithelium as well as swelling and vacuolization in the endothelial cells lining the glomerular tufts. Focal haemorrhages with tubular cystic dilation were observed in the corticomedullary portion. In accordance of our results Fernandes et al., (2016) demonstrated that experimental induction of DN in albino rats by STZ infusion led to classical signs of the disease such

as hyperglycemia. This finding demonstrates that maintenance of the hyperglycemic status favored the development of diabetic nephropathy evidenced by albuminuria and focal changes in renal histology. The devastating effect of systemic hyperglycemia on glomerular filtration makes endothelial cells unable to modulate glucose transport through the plasma membrane, leading to excessive accumulation of intracellular glucose. The high levels of intracellular glucose stimulate the synthesis of cytokines, such as transforming growth factor  $\beta$  and vascular endothelial growth factor. These factors are involved in microvascular endothelial lesions, increasing glomerular permeability to macromolecules and add to the changes in

glomerular hemodynamics (Fernandes et al., 2016).

Microscopic examination for the kidney in DN actuated group treated with AuNPs demonstrated that there was no histopathological change in the glomeruli and tubules at the cortex. Our histopathological comes about bolstered by Shaheen et al., (2016) who clarified that the possible mechanism of the defensive activity of AuNPs may due to the capacity of nanoparticles to reestablish the damage and increment activities of endogenous antioxidants enzymes in DN treated with AuNPs.

Furthermore, kidney tissue in DN actuated group treated with AgNPs demonstrated that there were vacuolization cells covering the tufts of glomeruli and cystic dilatation in a few tubules at the cortex. An investigation by Shaneen et al., (2016) demonstrated that histopathological changes in kidney treated with silver nanoparticles reestablish the typical structure through the capacity of silver nanoparticles to reduce the production of reactive oxygen and nitrite species, which don't evoke secretion of proinflammatory cytokines TNF- $\alpha$  and IL1- $\beta$ , making them appropriate candidates for nanomedicine .

Pancreatic tissues in DN prompted group demonstrated that there was atrophy with small size islands of Langerhans in diffuse way. In concurrences with Goud et al., (2015) the induction of DN by STZ demonstrated that STZ is cytotoxic to pancreatic  $\beta$ -cells and its impact could be found in the histology, the toxic activity of STZ includes its selective up take into  $\beta$ -cells through glucose transporter GLUT-2 show in the plasma membrane.

Pancreatic sections treated with gold nanoparticles demonstrated that the islands of Langerhans cells were decayed and absent as

per Al-Azzawie and Yaaqoob (2016) who demonstrated that AuNPs impact on pancreas by increased size of islets and hyperchromic nucleus which might be an indication of recovery. Indications of recovery of  $\beta$ -cells in the islets of Langerhans and diminished blood glucose have been accounted for the utilization of some metal nanoparticles (Al-Azzawie and A.Yaaqoob, 2016).

Based on our results, the present study shed the light on the promising hypoglycemic and anti-fibrotic effect of gold and silver nanoparticles on DN induced experimentally. Also, Its biochemical role in normalization blood glucose and serum insulin as well as kidney function biomarkers.

## CONCLUSION

. Based on our results, the present study shed the light on the promising hypoglycemic and anti-fibrotic effect of gold and silver nanoparticles on DN induced experimentally. Also, its biochemical role in normalization blood glucose and serum insulin as well as kidney function biomarkers was documented in this study.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

AH conceived and developed the idea of the paper and also wrote the manuscript. HB, performed animal treatments, biochemical analysis, tissue collection, and data analysis. All authors read and approved the final manuscript.

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