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Original Paper

Pancreatic Response to Gold Nanoparticles Includes Decrease of Oxidative Stress and Inflammation In Autistic Diabetic Model

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Key Words
AuNPs • ORAC • Antioxidant • Autism

Abstract
Background: Gold nanoparticles (AuNPs) have a wide range of applications in various fields. This study provides an understanding of the modulatory effects of AuNPs on an antioxidant system in male Wistar diabetic rats with autism spectrum disorder (ASD). Normal littermates fed by control mothers were injected with citrate buffer alone and served as normal, untreated controls controlin this study. Diabetes mellitus (DM) was induced by administering a single intraperitoneal injection of streptozotocin (STZ) (100 mg/kg) to the pups of (ND) diabetic group, which had been fasted overnight. Autistic pups from mothers that had received a single intraperitoneal injection of 600 mg/kg sodium valproate on day 12.5 after conception were randomly divided into 2 groups (n 2 7/group) as follow; administering single intraperitoneal injection of streptozotocin (STZ) ((100 mg/kg) to the overnight fasted autistic pups of (AD) autistic diabetic group. The treatment was started on the 5th day after STZ injection with the same dose as in group II and it was considered as 1st day of treatment with gold nanoparticles for 7 days to each rat of (group IV) treated autistic diabetic group(TAD) at a dosage of 2.5 mg/kg. b. wt. ***Results:*** At this dose of administration AuNPs, the activities of hepatic superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase were greater in group TAD compared with the control group ($P < 0.05$). Oxidised glutathione levels were lower ($P > 0.05$) in the liver of autistic diabetic AuNPs –supplemented rats, whereas reduced glutathione was markedly higher than in control rats, especially after administration of AuNPs. Moreover, the kidney functions in addition to the fat profile scoring supported the protective potential of that dose of AuNPs. The beta cells revealed euchromatic nuclei with no evidence of separation of nuclear membrane. ***Conclusions:*** Our results showed that AuNPs improved many of the oxidative stress parameters (SOD, GPx and, CAT), plasma antioxidant capacity (ORAC) and lipid profile relative to the other parameters. In addition to the apparent reversibility of the pancreatic B cell in group IV which may reflect the regenerative capacity of AuNPs.

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Introductions

Oxidative stress is associated with the diminished capacity of a biological system to counteract the overproduction or invasion of reactive oxygen species and other radicals. Because oxidative stress is the leading cause of DNA damage, genetic disorders, cancer, and many environmental pollution-related diseases, there is an urgent need for oxidative stress screening and prevention. Oxidative stress is enhanced in autism, a disorder with poorly understood aetiology and neurobiology. There is growing evidence that oxidative stress can cause autism in children [1]. Because oxidative stress plays a significant role in the neurobiology of autism [2, 3], targeting of oxidative stress is suggested as a therapeutic approach [2]. Superoxide dismutase (SOD) and GSH-Px levels are lower in autistic individuals than in controls [4, 5]. In addition, the concentrations of exogenous antioxidants, vitamins E and A, and lycopene in individuals with autism are insufficient [6].

Oxidative stress has also been implicated in the development of diabetes. The tripeptide glutathione and its oxidised form, glutathione disulphide (GSSG), form a redox potential maintenance system in all eukaryotic cells. Because glutathione efficiently protects DNA, proteins and lipid membranes from radical attacks, diminished levels of glutathione are a signal of oxidative stress and the increased vulnerability of a biological entity to environmental influences. An increased level of 3-nitrotyrosine, which is formed under oxidative stress in the presence of nitric oxide, has been found in diabetic patients [7]. In this investigation, methods for detecting oxidative stress biomarkers based on their interactions with gold nanoparticles (AuNP) are described. Specific maternal illnesses, conditions and treatments can result in adverse neurodevelopment outcomes in children [7]. Perinatal complications place an infant at significant risk for mental, neurological and behavioural disorders [8]. Maternal metabolic conditions may increase the risk of ASD. Maternal Type 2 diabetes, hypertension, and obesity have been identified as risk factors for ASD and other developmental disorders [7, 9]. Prenatal factors, such as advanced maternal (and paternal) age, bleeding or gestational diabetes, have been associated with the risk of ASD [10]. Elevated prenatal cortisol is known to negatively affect the behaviour of newborn children with increased irritability, attention and temperament problems [11]. Excess plasma cortisol levels have been implicated in the aetiology of comorbid illnesses associated with ASD, such as depression, anxiety, dyspepsia and migraine [12]. Furthermore, elevations in plasma cortisol and platelet serotonin levels have been observed in schizophrenic patients [12]. Hence, there is evidence to suggest that excess cortisol levels co-exist with serotonin-selective pathologies. One of the prenatal risk factors for ASD is gestational diabetes. During late pregnancy mothers can develop insulin resistance [13]. Gestational diabetes occurs in up to 14% of all pregnancies; estimates vary depending on the test criteria used [14]. Elevated cortisol levels have been measured in pregnant women with impaired glucose tolerance or gestational diabetes [15]. Gestational diabetes is a growing health concern with both short- and long-term outcomes for both mothers and their offspring [16]. Deterioration of glucose tolerance occurs in all women with the development of diminished peripheral sensitivity to insulin [17]. Normal pregnancy is characterised by insulin resistance and hyperinsulinemia, particularly during the third trimester, due to elevated metabolic stress on maternal lipids and glucose homeostasis [18]. Progesterone receptors expressed in pancreatic islet cells inhibit beta cell proliferation to reduce insulin secretion and glucose tolerance during pregnancy [16]. Foetal hyperglycaemia as an outcome of maternal hyperglycaemia can contribute to either increased or decreased birth weight [19]. Many prenatal risk factors for ASD can alter cortisol levels either directly or indirectly. Prenatal depression and psychological stress are associated with elevated cortisol levels, prematurity and low birth weights [20]. In mammals, glucocorticoids are central to foetal growth, tissue development and the maturation of various organs [21]. Normally, foetal physiological glucocorticoid levels are lower than the maternal levels [22]. Some children diagnosed with ASD or who have higher scores on ASD spectrum screening have low birth weights [23], potentially due to elevated cortisol levels present during the prenatal period. Furthermore, males are

more vulnerable to elevated maternal cortisol levels, as the placenta of female foetuses exhibit increased glucocorticoid inactivation and lower corticoid receptor densities than the placentas of males [24]; this difference may render and explain gender differences in the prevalence of ASD. The future widespread use of nanoparticles is likely to have an enormous impact on human disease, particularly diabetes. Therefore, it is essential to understand the effects of nanoparticles on the pancreas, which is one of the major organs affected by this disease. This study was undertaken to investigate the effect of AuNPs (50 nm) for 7 days on autistic rats with diabetes. Their effects on oxidative stress and antioxidant defense indices were investigated together with the electron microscopic study of the pancreas.

Materials and Methods

Ethical issues

All procedures in this study were carried out according to the National Guidelines on Animal Experimentation, and the protocol was approved by the ethical committee of the Faculty of Medicine of King Khalid University. All efforts were made to minimise animal suffering, and the minimum number of animals necessary to produce reliable scientific data was used.

Experimental animals

Wister-albino rats of both sexes weighing 160-180 g were obtained from the Laboratory Animal Unit of King Khalid University and housed in plastic cages with mesh grid floors for acclimatisation and mass breeding. The cages were thoroughly cleaned and the animals were examined daily. Clean tap water and rat feed were made available ad libitum. The temperature of the animal room was 33 ± 3°C with a 12 h:12 h light-darkness cycle. The animals remained uniformly healthy. Rats that became pregnant were isolated into solid floor maternity cages. Fine, sterilised wood shavings were provided as bedding and nesting material. Immediately after weaning (i.e., before sexual maturity), the offspring were transferred into new cages separated by sex to prevent mating before the experimental induction of pregnancy. This separation was conducted to ensure that the animals were virgins prior to inducing pregnancy.

Induction of pregnancy

At 90 days of age, 20 virgin rats (10 females and 10 males) produced from the previous mass breeding period were housed in 10 mating groups of monogamous pairs (1 female and 1 male per cage). At this age, the animals have reached sexual maturity and the vagina has opened. To ascertain successful mating, vaginas were examined every morning, and vaginal smears were obtained to detect whether sperm cells were present. In addition, the vaginas and cage floors were examined for the presence of cornified plugs. The presence of sperm cells in the vaginal smear or the presence of a cornified plug in the vagina or on the cage floor indicated successful mating and marked Day 1 of gestation. Gestating females were separated into maternity cages and constituted pregnant rats for the subsequent intraperitoneal injection of sodium valproate on day 12.5 after conception.

Animal treatment

Females were fed a standard diet and received a single intraperitoneal injection of 600 mg/kg sodium valproate on day 12.5 after conception. Administration of this dose to rats during embryogenesis has been shown to result in a maximum level of total VPA (900 μg/mL) in maternal plasma in less than 1 h, with a mean plasma elimination half-life of 2.3 h [25]. Control females were maintained on normal standard diet and injected with physiological saline at the same time (i.e., on day 12.5 after conception). Sodium valproate (Sigma) was dissolved in saline at a concentration of 250 mg/mL. Dams were housed individually and were allowed to raise their own litters. After an adaptation period of 1 wk to confirm autism by blood tests (results not shown), unmanipulated littermates fed by control mothers were injected with citrate buffer alone and served as untreated controls (Control) in this study. Diabetes mellitus (DM) was induced by administering a single intraperitoneal injection of streptozotocin (STZ) (100 mg/kg) in 0.1 mol/L citrate buffer (pH 4.5) [26] to individual pups fasted overnight; these comprised the diabetic group (ND). Because of the instability of STZ in aqueous media, the solution was made using cold citrate buffer (pH 4.5) immediately prior to

administration. A fasting blood glucose level above 11.1 mmol/L was considered diabetic and included in the analysis. Autistic male pups from mothers that had received a single intraperitoneal injection of 600 mg/kg sodium valproate on day 12.5 after conception were randomly divided into 2 groups (n 2 7/group) as follows. A single intraperitoneal injection of streptozotocin (STZ) (100 mg/kg) was administered to the fasted autistic male pups of the autistic diabetic group (AD). Treatment began on day 5 after STZ injection at the same dosage as administered to Group II. This constituted day 1 of treatment with gold nanoparticles, administered by intraperitoneal injection via a tuberculin syringe, at a dosage of 2.5 mg/kg. b. wt [27] for 7 days to each rat of the autistic diabetic treated group (TAD).

Oxygen radical absorbance capacity (ORAC)

The antioxidant capacity was measured as the oxygen radical absorbance capacity (ORAC method) [28]. This assay measures the oxidative degradation of fluorescein after being mixed with free radical generators such as azo-initiator compounds. The ORAC values were calculated and expressed in mmol Trolox equivalents/mg protein. Free radicals were produced using 2,29-azobis (2-amidinopropane) hydrochloride (AAPH), and the oxidation of the fluorescent indicator protein b-PE was measured. Both reagents were prepared in 75 mmol/L phosphate buffer (pH 7.0), and 50 mmol/L Trolox was used as the standard. The liver samples were homogenised in 4 volumes of phosphate buffer in a Thomas homogeniser (20 strokes) and centrifuged at 12,000 x g for 10 min at 48°C. The supernatant was deproteinised using 0.25 mol/L PCA and centrifuged at 16,000 3 g for 15 min. The supernatants were then stored at 280°C before analysis. The reaction was performed in 96-well microtiter plates and consisted of 170 mL of b-PE (80 mg/L) and 10 mL of diluted (1:1) sample incubated at 37°C for 15 min. The reaction was initiated by the addition of 20 mL of AAPH (240 mmol/L), and the fluorescence (emission 590 nm, excitation 530 nm) was recorded every 5 min until the reading had declined to 0.5% of the initial reading.

Plasma and liver samples were obtained for further analysis. Liver homogenates were prepared with 50 mmol/L Tris buffer containing 0.25 mol/L of sucrose pH 7.5. Liver homogenates were centrifuged at 100,000 3 g for 1 h at 4°C. Cytosol aliquots were collected and preserved at -80°C for enzymatic assay.

Antioxidant enzymes assays

Total SOD activity was determined following Spitz and Oberley [29]. The total SOD activity in each sample was calculated using a concurrently run SOD (Sigma Chemical) standard curve and expressed as U/ mg sample protein. Tissue GPx activity was measured following Flohe and Gunzler [30]. Catalase activity was measured following Aebi [31]. Total glutathione (GSH and oxidised glutathione, GSSG) was measured following Tietze [32]. The change in absorbance was monitored at 410 nm for 5 min, and GSH and GSSG levels were calculated using pure GSH and GSSG as standards.

Lipid peroxidation assays

Liver lipid peroxidation was assessed as the amount of thiobarbituric acid reactive substances (TBARS) produced following Tappel and Zalkin [33].

Lipid composition analysis

Following Bligh and Dayer [34]. Triglecerides were determined following Gottfried and Rosenberg [35] and total cholesterol was determined following Zlatkis et al. [36].

Transaminase assay

[37]. Urea and creatinine levels in the plasma were estimated by the method described earlier [38].

Glucose, glycogen, cortisol and serotonin estimation

Blood samples were obtained immediately to estimate blood glucose level with a 201_ glucose meter (Hemocue Ltd., Sheffield, U.K.), measured in mmol/L. Hepatic glycogen concentration (µmol/g of liver) was measured using the enzymatic procedure of Gire [39]. Plasma cortisol levels were determined according to the competitive protein binding procedure of Dalle and Delost [40] and expressed in µmol/ L. Plasma serotonin level was analysed using high performance liquid chromatography (HPLC) as described previously [41].

Transmission electron microscopic study

Small samples from the pancreas of each experimental group on PND 21 were immediately fixed in 3% phosphate-buffered glutaraldehyde (pH = 7.4; 4°C) for 2 h. The tissues were post-fixed in 1% aqueous osmium tetraoxide in an appropriate buffer for 1 h and embedded in Epon. Ultrathin sections (80-100 nm) were prepared and stained with uranyl acetate and lead citrate.

Statistical analysis

The results are expressed as means ± SD. ANOVA was used to evaluate differences between multiple groups, and comparisons between the means of the treated groups and the control group were made using Dunnett's test. Differences were considered significant at $P < 0.05$.

Results

Antioxidant status and oxidative stress

The biomarkers of antioxidant status and oxidative stress are summarised in Table 1. The concentrations of antioxidant enzymes indicated an activation of these enzymes in group TAD. SOD and GPx values were higher in the group treated with gold nanoparticles than in the other groups. Autistic diabetic rats administered gold nanoparticles had significantly ($P < 0.05$) higher hepatic SOD, GPx, compared with both the autistic diabetic group and the diabetic group, suggesting that AuNPs were very effective at increasing the antioxidantstatus in the liver. No significant differences in catalase activity were observed between normal diabetic group and the autistic diabetic group after treatment with gold nanoparticles. Finally, antioxidant capacity (ORAC) was significantly higher in group TAD than in the other groups. This result is in agreement with the higher SOD, GPx and GR values found in this AuNP-supplemented rats (Table 1) .

Lipid peroxidation

TBARS levels in the liver measure the liver's susceptibility to lipid peroxidation. As shown in Figure 1, we observed increased levels of TBARS in all groups relative to the control group, with the lowest levels observed in AuNPs –treated rats. These results suggest that the diabetic and autistic diabetic groups were more susceptible to lipid peroxidation than group TAD and that gold nanoparticles increased the values of antioxidant enzymes relative to those of group ND and group AD.

Lipid profile

The mean LDL values indicated higher oxidation in the diabetic group and autistic diabetic group than in controls but the treated autistic diabetic group showed no significant differences from the control groups (Fig. 2). TG and HDL values were within the reference ranges in all groups relative to controls, whereas CHOL concentrations were higher in both groups ND and AD, but did not differ significantly between group TAD and controls.

Table 1. Oxygen radical absorbance capacity (ORAC) and oxidative stress biomarkers in the liver of Wistar rats of different treatment groups. Values represent means ± SEM; values in the same row with different superscripts are statistically significant $p < 0.05$

Parameters	Control	ND	AD	TAD
GSH (mmol/mg protein	11.1±2.3^a	6.9±2.2^b	5.1±2.6^b	12.7±1.9^a
GssG (mmol/mg protein	1.8±0.6^a	2.2±0.1^b	2.5±0.3^b	1.1±0.3^a
Catalase mmol/ (mg protein)	12.1±2.1^a	8.3±2.5^b	7.1±3.2^b	9.8±2.1^b
SOD U/mg protein	25.1±2.6^a	13.9±3.6^b	15.2±2.7^b	20.7±3.8^a
Glutathione Peroxidase nmol/ (min_mg protein)	22.3±1.5^a	18.1±2.6^b	16.3±5.1^b	20.1±2.7^a
Glutathione Reductase nmol/ (min_mg protein)	7.2±0.2^a	5.1±0.1^b	5.2±0.5^b	6.0±0.2^b
ORAC (mmol Trolox/mg Protein)	20.5±1.3^a	14.5±1.7^b	16.1±2.1^b	21.8±2.1^a

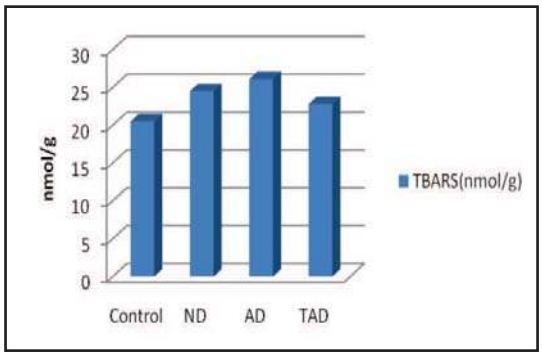

Fig. 1. Lipid peroxidation products (TBARS) in the liver of rats of different treatment groups.

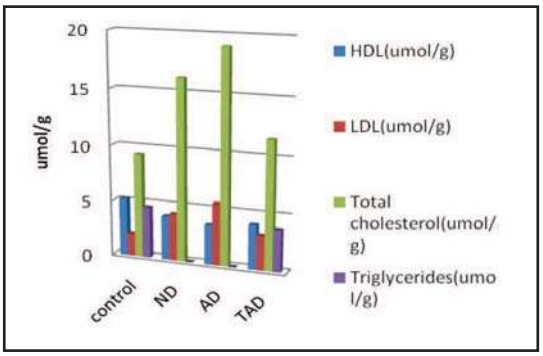

Fig. 2. Influence of gold nanoparticles on the lipid profile of different treatment groups.

Table 2. Comparison of glucose and glycogen in different treatment groups. Values represent means ± SEM; values in the same row with different superscripts are statistically significant $p < 0.05$

Parameters	Control	ND	AD	TAD
Glucose (mmol/L)	6.22±1.3[a]	15.12±6.2[b]	14.01±2.2[b]	5.1±5.2[a]
Glycogen (µmol/g)	150±0.3[a]	125±0.6[a]	165±0.6[b]	135±0.1[a]

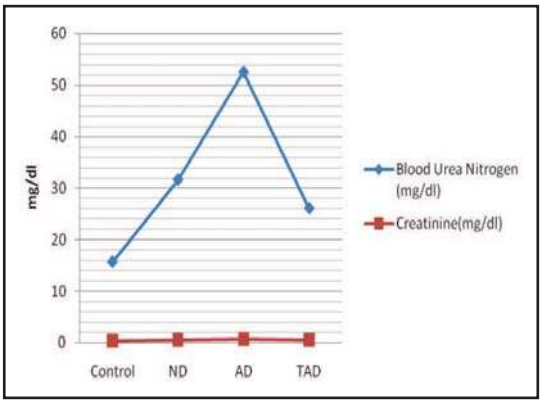

Fig. 3. Comparison between blood urea nitrogen (BUN) and creatinine in different treatment groups.

Glycaemia control and glycogen accumulation

Blood glucose was significantly elevated in autistic rats (results not shown). Furthermore, the increases in glucose level observed in groups ND and AD were not significantly different from those of the autistic rats, and did not differ among groups. . Nanoparticle treatment in group TAD significantly decreased blood glucose to close to normal levels in the control group (Table 2). Glycogen accumulation in the livers of autistic diabetic pups was high relative to normal control pups; however, a significant decrease in glycogen levels was observed in normal diabetic pups and treated autistic diabetic pups relative to the highest levels in autistic diabetic pups.

Cortisol and serotonin estimation

Plasma cortisol levels increased in autistic diabetic group but remained unchanged in the other groups. This increase in diabetic pups was accompanied by a significant reduction in cortisol levels after treatment with gold nanoparticles relative to controls. When comparing serotonin levels among all groups, significant differences were observed between both the diabetic and autistic diabetic groups and the control group ($P < 0.05$).However, no significant differences in serotonin levels between autistic diabetic pups treated with gold nanoparticles and control groups were observed. The reduction in cortisol and serotonin concentrations following nanoparticle administration indicates its potential for use in studies of autism and diabetes.

Creatine and BUN estimation

As shown in Figure 3,diabetic autistic rats treated with gold nanoparticles in group TAD showed decreased levels of creatine and uric acid relative to controls; however, the highest

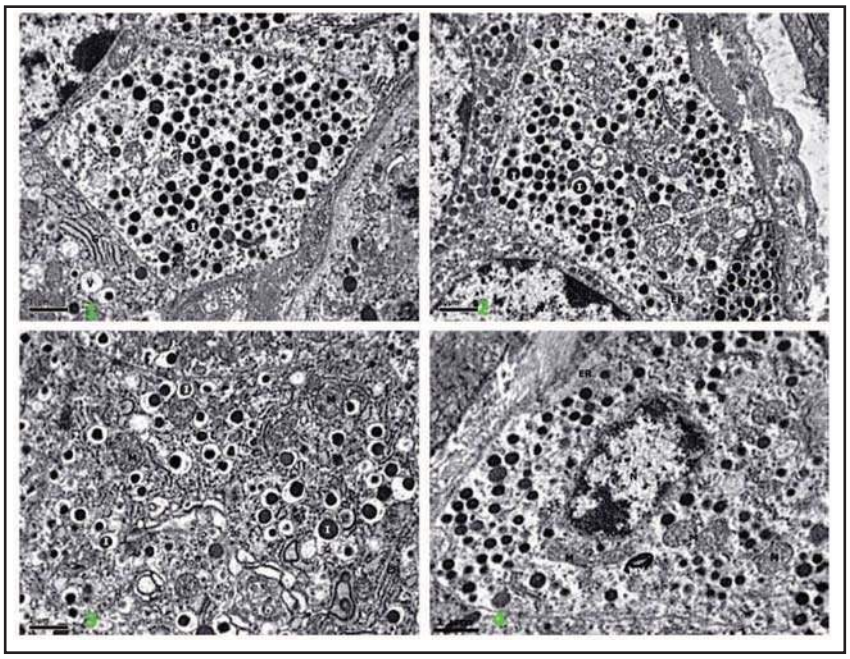

Fig. 4. Control pancreatic cells (group I) exhibiting normal beta cells Islets of Langerhans with numerous electron dense secretory granules (I), numerous mitochondria (M), rough endoplasmic reticulum (ER), Golgi apparatus (G), a few myelin Fig. (MY), secretory vacuoles (V) and the euchromatic nucleus (N). Scale bar = 1 µm.

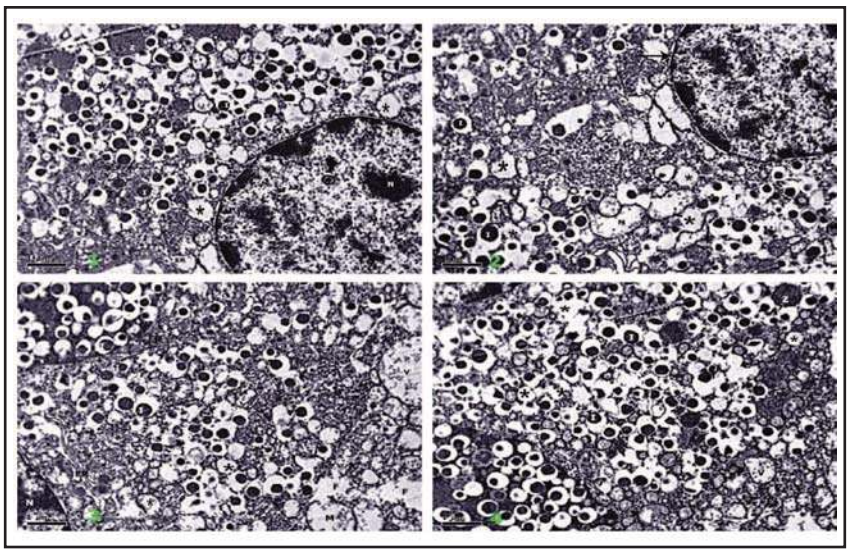

Fig. 5. Normal diabetic (group II) shows euchromatic nucleus (N), missing of insulin containing granules (I) and Zymogen granules(Z), few vacuoles in the beta cells (asterisk) and increase of secretory vacuoles (V) with slight fusion (F). Slight separation in nuclear membrane (arrow) and crystolysis of the mitochondria (M). Scale bar = 1 µm.

increases of creatine and uric acid relative to control rats were recorded in autistic diabetic group. These results reveal the restorative effect of gold nanoparticles on renal function.

TEM findings

Control group. The islets of Langerhans were normal and were comprised primarily of beta cells. The cytoplasm of these cells contained numerous electron-dense secretory granules (insulin-containing granules). The latter are surrounded by wide lucent halos and numerous mitochondria, rough endoplasmic reticulum, Golgi apparatus, few myelin Fig. and a euchromatic nucleus (Fig. 4).

*Normal diabetic group.*The beta cells in the islets of Langerhans of the pancreas revealed euchromatic nuclei and a decreased number of insulin-containing granules, in addition to few vacuoles in the beta cells and an increase in the number of secretory granules with fusion. Few cases showed mildly dilated rough endoplasmic reticulum, crystolysis of the mitochondria and separation of the nuclear membrane (Fig. 5).

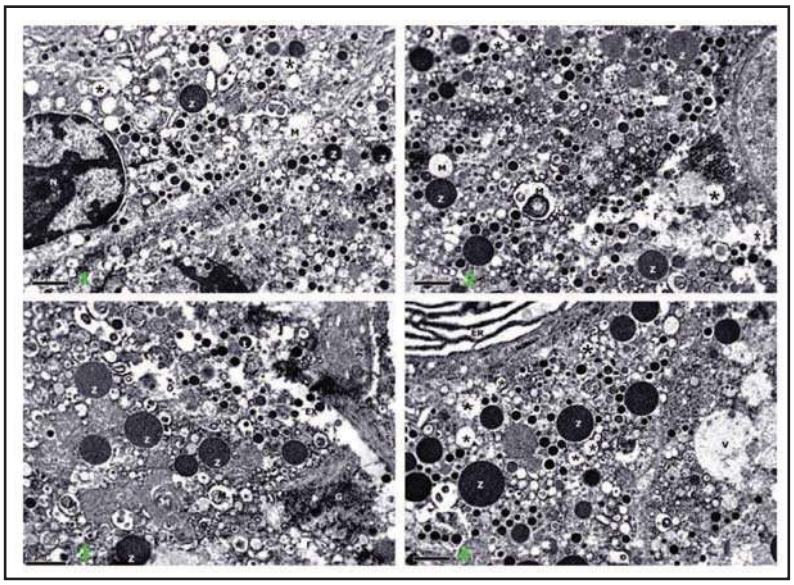

Fig. 6. Autistic diabetic (group III) shows: vacuolation in the beta cells in islets of Langerhans (asterisk), depletion of secretory granules (I), fusion of some vacuoles (F), crystolysis of the mitochondria (M), dilation of the rough endoplasmic reticulum (ER), pyknotic nuclei (N), separation of nuclear membrane(arrow), focal accumulation of glycogen granules (G) and few lipid droplets (L), numerous myelin Fig. (MY) and zymogene-like granules (Z). Scale bar = 1 µm.

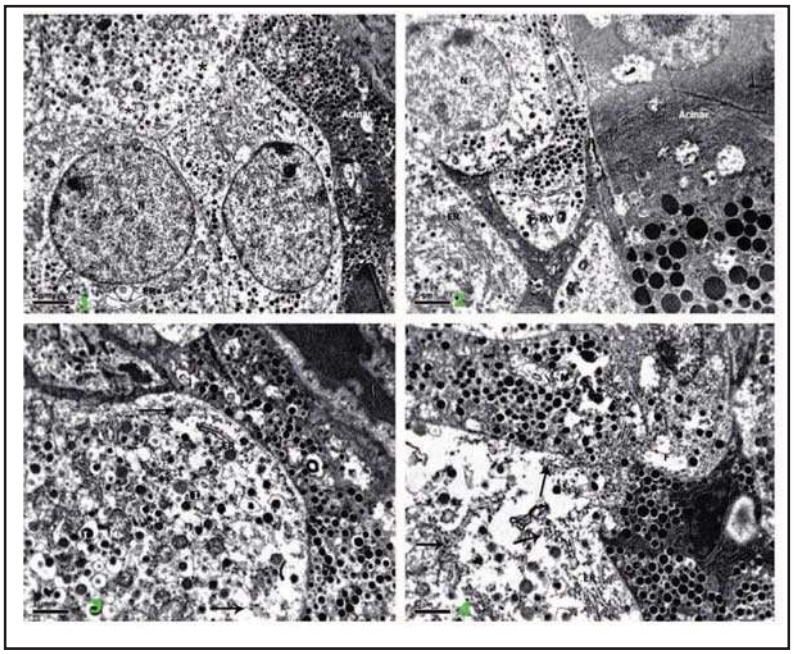

Fig. 7. Treated autistic diabetic(group IV) exhibiting euchromatic nuclei (N), a reduction in insulin containing granules (asterisk), normal endoplasmic reticulum (ER) and secretory vacuoles (V), fusion of some vacuoles (F), normal mitochondria (M) and the presence of gold nanoparticles in the cytoplasm (arrows). Scale bar = 2 and 1 µm.

Autistic diabetic group. The degree of cell damage caused by streptozotocin varied, even within the same acinus. A marked increase in zymogen granules was confirmed in damaged cells; these become dispersed throughout each cell. Approximately 80-90 granules were observed in severely damaged cells; i.e., approximately three times the number observed in electron micrographs of normal cells. The beta cells in the islets of Langerhans showed vacuolation and depletion of secretory granules, fusion of some granules and the formation of large secretory vacuoles. Other changes involved the mitochondria and the ER. Crystolysis of the mitochondria, dilation of the rough endoplasmic reticulum and pyknotic nuclei were observed. In some cases, the nuclear membrane exhibited strongly separated condensed chromatin. Glycogen granules and a few lipid droplets accumulated in the affected cells with numerous myelin Fig. and zymogene-like granules, particularly adjacent to the acinar cells (Fig. 6).

Treated autistic diabetic group. The gold nanoparticles-treated group showed some degenerative changes, although not as severe as observed in group ND&AD. The beta cells

revealed euchromatic nuclei with no evidence of separation of nuclear membrane, a slight decrease in the number of insulin-containing granules with increased secretory vacuoles and fusion. The rough endoplasmic reticulum and mitochondria were normal except in some cases where dilation of the endoplasmic reticulum was observed. Gold particles were also observed scattered throughout the cytoplasm of these cells (Fig. 7).

Discussion

Nanotechnology is undergoing a broad expansion in many areas benefitting humans. The promising potential of nanotechnology has increased interest in investigating the anti-oxidative and anti-hyperglycemic autistic activity of gold nanoparticles in the diabetic autistic model. The goal of our investigation was to use nontoxic, biologically synthesised gold nanoparticles, sized 50 nm, to overcome the drawbacks of the in vivo system of autistic diabetic rats. Recently, intraperitoneal injection of gold nanoparticles was investigated by Lasagna-Reeves et al. [42]. They found a low level of toxicity at a dosage range of 320–3200 μg/kg/day. Zhang et al. [43] observed that gold nanoparticles are less toxic when delivered by intraperitoneal injection than by oral administration at a dose of 1100 μg/kg. Nanoparticle size is a key factor in biological responses to nanoparticles; smaller particles tend to be more toxic than larger ones. Exposure to gold nanoparticles (average diameter 5.3 ± 1 nm) produced oxidative stress within 24 h in Mytilus edulis [44]. At sizes larger than 5 nm, the general assumption is that gold is chemically inert. However, the chemical reactivity of gold particles at diameters of less than 3 nm is most likely different than it is for larger gold nanoparticles [45]. Zidki et al. [46] reported a potential radical scavenging property of gold nanoparticles against alkyl radicals. Zhang et al. [47] also observed a reduction in the EPR signal (indication of radical scavenging) following the interaction of free radicals with 15 nm gold nanoparticles. Barathmanikanth et al. [48] described the potential of gold nanoparticles as a therapeutic remedy in diabetic complications and as an anti-oxidative agent; these benefits arise through the scavenging of free radicals and the creation of a sustained control over hyperglycemic conditions, suggesting inhibition of the formation of reactive oxygen species.

Oxidative stress has been suggested as the causative factor in aging [49]and in many diseases such as diabetes, cardiovascular disease, cancer and autism spectrum disorders (ASD [50]. Oxidative stress is enhanced in autism [20] and plays a significant role in its neurobiology [21, 22]; therefore, targeting of oxidative stress is suggested as a therapeutic approach [21]. The antioxidant enzymes superoxide dismutase (SOD) and GSH-Px are lower in autism than in controls [23, 24]. Among the biomarkers of oxidative stress are small biomolecules such as glutathione GSH and SOD, which are depleted in the presence of organic radicals and peroxides [51]. Autism is a disorder characterised by enhanced oxidative stress [52], decreased levels of the antioxidant enzymes SOD and GSH-Px [53], brain Inflammation, apoptosis [54], increased the levels of the proinflammatory cytokines TNF-a and IL-6 [54] and impaired mitochondrial energy production [55]. Therefore, gold nanoparticles may at least partially improve some of the symptoms of autism(Table 1,2&3). However, it should be emphasised that some ROS have roles as second messengers [56].

In the present study, a statistically significant increase in the levels of GSH, SOD and GPx in the diabetic autistic rats treated with AuNPs relative to controls was found(Table 1). This result was due to the significant decrease in lipid peroxidation and ROS generation in diabetic autistic rats treated with AuNPs relative to controls, suggesting that AuNPs prevent the disruption of organs by protecting lipids from peroxidation by ROS under hyperglycaemic autistic conditions. The enhancement of AuNP 5 nm by GSH molecules is attributed to the size increase of AuNP due to the ligand exchange and interparticle interactions leading to AuNP assembly. The zwitterionic forces are dominant, although the main forces in the GSH-induced assembly are H bonding [57]. In contrast, our results revealed that AuNPs did not alter the level of catalase activity (Table 1), potentially due to reactive oxygen species (ROS),

Table 3. Comparison of cortisol and serotonin in different treatment groups. Values represent means ± SEM; values in the same row with different superscripts are statistically significant $p < 0.05$

Parameters	Control	ND	AD	TAD
Cortisol (µmol/L)	2.6±0.2[a]	4.6±0.5[a]	7.3±0.2[b]	3.1±0.7[a]
Serotonin (ng/mL)	98±0.5[a]	123±0.1[b]	178±0.3[b]	96±0.6[a]

including H2O2, that affect the living system. ROS can oxidise cell components and lead to inactivation of certain enzymes; in addition, they are known to be involved in oxygen sensing and signal transduction as secondary messengers [56]. As catalase activity in group IV did not differ from controls, H2O2 may not have been generated in sufficient amounts by the AuNPs to alter catalase activity. Elevated glucose levels are associated with an increased production of ROS by several different mechanisms [58]. In addition, the process of glucose auto-oxidation generates superoxide, which is associated with the formation of glycated proteins in the plasma of diabetic patients [59]. The interaction of advanced glycation end products with corresponding cell surface receptors stimulates ROS production and decreases intracellular glutathione levels [60]. The increase in ROS production contributes to the development of diabetic complications, such as atherosclerosis, and other vascular complications, such as autism [60]. However, if the initial increase in ROS in response to oxidative stress conditions is relatively small, the antioxidative response may be sufficient to compensate for the increase in ROS and to reset the original balance between ROS production and ROS scavenging capacity. The physiological manifestations of redox regulation typically involve a temporary increase and/or a temporary shift of the intracellular thiol/disulphide redox state toward more oxidative conditions. However, in the autistic diabetic group, ROS production is stronger and more persistent, ROS generated by high glucose levels play a vital role in the development of diabetic complications [56]. and the antioxidative response may not be sufficient to reset the system to the original level of redox homeostasis, as it may now be associated with higher ROS concentrations and different levels of free amino acids and/or different patterns of gene expression. Such a shift to more oxidative conditions has occurred in the autistic diabetic rats, implying that a pro-oxidative shift is an overtly pathological condition.

In addition, hyperglycemia enhances cell-mediated low-density lipoprotein (LDL) peroxidation in endothelial cells [61]. Treatment with antioxidants ameliorates diabetic complications, including the dysfunction of endothelial cells and increased platelet aggregation [61]. Recently, the anti-glycation activity of gold nanoparticles and their biocompatibility has made them desirable for ophthalmological implications [62]. In our investigation, the autistic diabetic-treated group did not show significant differences in LDL relative to the control group(Fig. 2).. Furthermore, the total cholesterol and triglycerides levels in group IV were restored to near-normal levels, thus yielding lipid functioning similar to that of the control group. Further, AuNPs administered at a dosage of 2.5 mg/kg. b. wt significantly decreased the blood glucose and urea levels group TAD relative to controls, reflecting that the absorbed nanoparticles in the systemic circulation are able to be excreted by the kidney.

Serotonin is taken up into the beta cells of the pancreas, where it is stored in granules that contain insulin to modify insulin release [63]. Disturbances in brain serotonin levels can affect the serotonergic system, leading to incorrectly connecting neural circuits [64]. Changes in serotonergic function and signalling have been found to be associated with ASD [65]. In addition, inflammatory cytokines, such as the interleukins (IL-1, IL-6) and TNF-α acting at the pituitary and adrenocortical levels, stimulate cortisol formation [66].

Excessive plasma levels of cortisol increase the expression of the SERT (Serotonin reuptake transporter), altering serotonin levels and modify prenatal neuronal development in children diagnosed with ASD. ASD is a disorder, and SERT has been the focus of much research due to its prominent role in serotonin homeostasis. SERT is encoded by the SLC6A4 (Solute carrier family 6 (neurotransmitter transporter, serotonin, member 4) gene. Several gene variants of SLC6A4 that are associated with ASD alter the structure, function or

expression of SERT [67]. Hence, in our investigation, there is evidence to indicate that excess cortisol levels co-exist with serotonin-selective pathologies, as ASD and treatment with AuNPs in group TADcaused no significance changes in either cortisol or serotonin relative to controls (Table 3). Gold nanoparticles (AuNPs) can easily enter cells [68], and the finding that amine and thiol groups bind strongly to AuNP has led to their surface modification with amino acids and proteins for biomedical applications [69].

In the present study, we demonstrated that Wistar-albino rats given AuNPs are a suitable model for the treatment of autistic diabetic rats exhibiting some aspects of the metabolic syndrome, such as oxidative stress, insulin resistance, hypertriglyceridemia and ROS damaging effects. To date there have been no published trials demonstrating the effective treatment of diabetic autistic rats using AuNPs. Our treatment modalities have been directed toward the reduction of oxidative stress, the improvement of glucose levels, lipid-lowering agents, and hepatoprotective drugs. In the present study, we also used electron microscopy of the pancreas and investigated the effects of AuNP treatment on diabetic rats with autism. It has been suggested that islet cells may arise from exocrine cells [46] [63]. Our observations of granules in the Golgi zone of beta cells of diabetic rats suggest that the synthesis of some secretory material continues to take place in the beta cell. If these microgranules are precursors or intermediates of insulin, the mode of action of streptozotocin may be to block synthesis of the hormone or to interfere with the release of the immature granules from the Golgi zone [26]. The present study revealed ultrstructural changes after streptozotocin injection, as evidenced by destructed beta cells. Moreover, autism elicited significant morphological changes in diabetic rats through the severe injury of pancreatic beta cells, including a decrease in islets cell numbers (results not shown) and cell damage. Furthermore, in our study diabetic autistic rats selectively destroyed and rapidly accumulates in beta cell that shows experimental models of pancreatic damage with structural and functional alterations such as disorganisation of pancreatic architecture, and depletion of insulin- producing cells together with, crystolysis of the mitochondria, dilation of the rough endoplasmic reticulum and pyknotic nuclei. The cytotoxic action of autism is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentrations, leading to the rapid destruction of beta cells [70]. Our study shows that AuNPs increase bloodglucose levels in diabetic autistic rats. The apparent and at least partial reversibility of islet-cell damage in group TAD during the period when exocrine tissue was still intact indicates that AuNps may be useful not only in the study of diabetes but also in the study of autism for the investigation and regeneration of beta cells.

Furthermore, it has been found that oxidative stress (as observed in group ND &AD) is associated with decreased insulin biosynthesis and secretion, which is the main aetiology of glucose toxicity. Indeed, it was suggested that the pancreas might be more susceptible to oxidative stress than other tissues and organs because pancreatic islet cells show extremely weak manifestation of antioxidative enzymes [71, 72]. It is evident that oxidative stress plays a key role in insulin resistance and beta cell dysfunction through their ability to activate stress-sensitive signalling pathways [71]. Increases in intracellular glucose lead to an abundance of electron donors generated in the Krebs cycle. These drive the inner mitochondrial membrane potential upward; a state associated with mitochondrial dysfunction and increased ROS production [73]. Degirmenci et al. [74] reported a decrease in the secretory granules of beta cells, vacuolisation and the swelling of mitochondria after streptozotocin administration. The semiquantitative evaluation of islet ultrastructure after streptozotocin injection showed signs of both necrotic and apoptotic cell death and disturbances in the insulin secretory pattern during and after a streptozotocin perfusion [75]. Streptozotocin induces damage to beta cell DNA mitochondria and plasma membranes [76]. Vacuolation was one of the structural indications of the permeability disorders of the membranes, which results in the enhanced transport of water and electrolytes into the cell. The permeability disorder might be attributed to many cellular membrane insults caused by reactive oxygen species mediating the formation of lipid peroxides, which ultimately generate self-sustaining

lipid peroxidation [77]. It has been reported that AuNps 50 nm scavenges oxygen free radicals, inhibits lipid peroxidation and protects cellular macromolecules, including DNA, from oxidative damage [44]. The authors also note that size particles do not accumulate in the kidney, stating that although these size ranges provide general clearance mechanisms, other physical parameters, clinical significance, and the long-term persistence of gold nanoparticles that simultaneous affecting NP movement play a significant role in their distribution [27]. Our results corroborate those of previous studies demonstrating that gold nanoparticles of approximately 3, 5, 50 and 100 nm do not show signs of toxic effects [78].

Conclusions

In conclusion, under the conditions of this study, 50-nm-sized gold AuNPs were non-toxic and produced no systemic or local adverse effects at the given dose. We conclude that the low production costs and relative ease of modifying nanogold make it a feasible for future biomedical applications. However, they are easily inactivated by complexation and precipitation, which may limit their desired function in human systems.

Abbreviations

Superoxide dismutase (SOD); Glutathione peroxidase (GPx); Oxygen radical absorbance capacity (ORAC); Total glutathione (GSH); Oxidized glutathione (GSSG); Blood urea nitrogen (BUN); Autism spectrum disorder (ASD); Gold nanoparticles (NPs); Triglyceride (TG); Low density lipoprotein(LDL); High density lipoprotein(HDL); Serotonin reuptake transporter(SERT); Transmission electron microscopic (TEM); Thiobarbituric acid reactive substances (TBARS); Normal diabetic(ND); Autistic diabetic(AD); Treated autistic diabetic(TAD); Endoplasm reticulum(ER); Tumor necrosis factor-alpha (TNF-alpha); Interleukin-1 (IL-1); Interleukin-1 (IL-6).

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Disclosure Statement

None declared.

References

1 Palmieri L, Persico AM: Mitochondrial dysfunction in autismspectrum disorders: cause or effect? Biochim Biophys Acta 2011;1797:1130-1137.
2 Villagonzalo KA, Dodd S, Dean O, Gray K, Tonge B, Berk M: Oxidative pathways as a drug target for the treatment of autism. Expert Opin Ther Targets 2010, 14:1301-1310.
3 Ghanizadeh A: Oxidative stress may mediate association o f stereotypy and immunity in autism, a novel explanation withclinical and research implications. J Neuroimmunol 2011, 232:194-195.

4 Meguid NA, Dardir AA, Abdel-Raouf ER, Hashish A: Evaluation of oxidative stress in autism: defective antioxidantenzymes and increased lipid peroxidation. Biol Trace Elem Res 2011, 143:58-65.
5 Mostafa GA, El-Hadidi ES, Hewedi DH, Abdou MM: Oxidative stress in Egyptian children with autism: relation toautoimmunity. J Neuroimmunol 2010;219:114-118.
6 Krajcovicova-Kudlackova M, Valachovicova M, Mislanova C, Hudecova Z, Sustrova M, Ostatnikova D: Plasma concentrations of selected antioxidants in autistic children and adolescents. Bratisl. Lek Listy 2009;110:247-250.
7 Leonard H, De-Klerk N, Bourke J, Bower C: Maternal health in pregnancy and intellectual disability in the offspring: a population-based study. Ann Epidemiol 2006;16:448-454.
8 Wilkerson DS, Volpe AG, Dean RS, Titus JB: Perinatal complications as predictors of infantile autism. Int J Neurosci 2002;112:1085-1098.
9 Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, Hertz-Picciotto I: Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics 2012;129:e1121-e1128.
10 Gardener H, Spiegelman D, Buka SL: Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiatry 2009;195:7-14.
11 Reynolds RM: Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis–2012 Curt Richter award Winner. Psychoneuroendocrinology 2013;38:1-11.
12 Cowen PJ: Cortisol, serotonin and depression: all stressed out? Brit J Psychiatry 2002;180:99-100.
13 Gabbe SG: Gestational diabetes mellitus. N Engl J Med 1986;315:1025-1026.
14 American Diabetes Association: Gestational diabetes mellitus. Diabetes Care 2004;27:S88-S90.
15 Ahmed SA, Shalayel MH: Role of cortisol in the deterioration of glucose tolerance in Sudanese pregnant women. East Afr Med J 1999;76:465-467.
16 Zhang C, Ning Y: Effect of dietary and lifestyle factors on the risk of gestational diabetes: review of epidemiologic evidence. Am J Clin Nutr 2011;94:1975S-1979S.
17 Kuhl C: Glucose metabolism during and after pregnancy in normal and gestational diabetic women. 1. Influence of normal pregnancy on serum glucose and insulin concentration during basal fasting conditions and after a challenge with glucose. Acta Endocrinol (Copenh) 1975;79:709-719.
18 Buchanan TA, Xiang AH: Gestational diabetes mellitus. J Clin Invest 2005, 115:485-491.
19 Ornoy A: Prenatal origin of obesity and their complications: gestational diabetes, maternal overweight and the paradoxical effects of fetal growth restriction and macrosomia. Reprod Toxicol 2011;32:205-212.
20 Urizar GG Jr, Munoz RF: Impact of a prenatal cognitive-behavioral stress management intervention on salivary cortisol levels in low-income mothers and their infants. Psychoneuroendocrinology 2011;36:1480-1494.
21 Seckl JR, Holmes MC: Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. Nat Clin Pract Endocrinol Metab 2007;3:479-488.
22 Beitins IZ, Bayard F, Ances IG, Kowarski A, Migeon CJ: The metabolic clearance rate, blood production, interconversion and transplacental passage of cortisol and cortisone in pregnancy near term. Pediatr Res 1973;7:509-519.
23 Pinto-Martin JA, Levy SE, Feldman JF, Lorenz JM, Paneth N, Whitaker AH: Prevalence of autism spectrum disorder in adolescents born weighing < 2000 grams. Pediatrics 2011;128:883-891.
24 Clifton VL, Murphy VE: Maternal asthma as a model for examining fetal sex-specific effects on maternal physiology and placental mechanisms that regulate human fetal growth. Placenta 2004;25:S45-52.
25 Binkerd PE, Rowland JM, Nan H, Hendricks AG: Evaluation of valproic acid (VPA) developmental toxicity and pharmacokinetics in Sprague-Dawley rats. Fundam Appl Toxicol 1988;11:485-493.
26 Ito M, Kondo Y, Nakatani A, Naruse A: New model of progressive non-insulin-dependent diabetes mellitus in mice induced by streptozotocin. Biol Pharm Bull 1999;22:988-989.
27 Selvaraj B, Kalimuthu K, Muthuirulappan S, SureshBabu R, Hyung-seop Y, SooHyun E, Sangiliyandi G: Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. J Nanobiotechnology 2010;8:16.
28 Cao G, Booth SL, Sadowski JA, Prior RL: Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables. Am J Clin Nutr 1998;68:1081-1087.
29 Spitz DR, Oberley LW: An assay for superoxide dismutase activity inmammalian tissue homogenates. Anal Biochem 1989;179:8-18.

30 Flohe L, Gunzler WA: Assays of glutathione peroxidase. Methods Enzymol 1984;105:114-119.
31 Aebi H: Catalase in vitro. Methods Enzymol 1984;105:121-127.
32 Tietze F: Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 1969;27:502-522.
33 Tappel AL,Zalkin H: Inhibition of lipide peroxidation in mitochondria by vitamin E. Arch Biochim Biophys 1959;80:333-336.
34 Bligh EG,Dyer WJ: A rapid method of lipid extraction and purification. Can J Biochem Phsiol 1959;37:911-917.
35 Gottfried SP, Rosenberg B: Improved manual spectrophotometric procedure for determination of serum triglycerides. Clin Chem 1973;19:1077-1078.
36 Zlatkis A,Zak B,Boyle AJ: Anewmethod for the direct determination of serum cholesterol. J Lab Clin Med 1953;41:486-492.
37 Calzyme Laboratories [http://www.calzyme.com/catalog/acidphos.html]
38 Calam RR: Specimen processing separator gels: an update. J Clin Immunoassay 1988;11:86-90.
39 Gire P: Contribution a 1'6tude du determinisme des viandes a coupe sombre chez le mouton: 'facteurs de mobilisation du glycogène musculaire pendant le stress de transport. Th. 3e; Cycle, Clermont-Ferrand, 1976, 147 pp.
40 Dalle M, Delost P: Plasma and adrenal cortisol concentrations in foetal, newborn and mother guinea-pigs during the perinatal period. J Endocrinol 1976;70:207-214.
41 Kluge H, Bolle M, Reuter R, Werner S, Zahlten W, Prudlo J: Serotonin in platelets: comparative Analyses using New enzyme immunoassay and HPLC Test Kits and the traditional fluorimetric procedure. J Lab Med 1999;23:360-364.
42 Lasagna-Reeves C, Gonzalez-Romero D, Barria MA, Olmedo I, Clos A, Sadagopa Ramanujam VM, Urayama A, Vergara L, Kogan MJ, Soto C: Bioaccumulation and toxicity of gold nanoparticles after repeated administration in mice. Biochem Biophys Res Commun 2010;393:649-655.
43 Zhang XD, Wu HY, Wu D, Wang YY, Chang JH, Zhai ZB, Meng AM, Liu PX, Zhang LA, Fan FY: Toxicologic effects of gold nanoparticles in vivo by different administration routes. Int J Nanomedicine 2010;5:771-781.
44 Tedesco S, Doyle H, Blasco J, Redmond G, Sheehan D: Oxidative stress and toxicity of gold nanoparticles in Mytilus edulis. Aquat Toxicol 2010;100:178-186.
45 Tsoli M, Kuhn H, Brandau W, Esche H, Schmid G: Cellular uptake and toxicity of Au55 clusters. Small 2005;1:841-844.
46 Zidki H, Cohen D, Meyerstein L: Reactions of alkyl-radicals with gold and silver nanoparticles in aqueous solution Phys. Chemistry Chem Phys 2000;8:3552-3556.
47 Zhang AB, Levanon H, Fessenden RW, Meisel D: On the interactions of free radicals with gold nanoparticles. J Am Chem Soc 2003;125:7959-7963.
48 Barathmanikanth S, Kalishwaralal K, Sriram M, Pandian SR, Youn HS, Eom S, Gurunathan S: Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. J Nanobiotechnol 2010;8:16.
49 Carlo MD, Loeser RF: Increased oxidative stress with aging reduces chondrocytesurvival: correlation with intracellular glutathione levels. Arthritis Rheum 2003;48:3419-3430.
50 James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DJ, Cutler P, Bock K, Boris M, Bradstreet JJ, Baker SM, Gaylor DW: Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet B Neuropsychiatr Genet 2006;141B:947-956.
51 Droge W: Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
52 Ghanizadeh A: Malondialdehyde, Bcl-2, superoxide dismutase and glutathione peroxidase may mediate the association of sonic hedgehog protein and oxidative stress in autism. Neurochem Res 2012;37:899-901.
53 Meguid NA, Dardir AA, Abdel-Raouf ER, Hashish A: Evaluation of oxidative stress in autism: defective antioxidant enzymes and increased lipid peroxidation. Biol Trace Elem Res 2011;143:58-65.
54 Malik M, Sheikh AM, Wen G, Spivack W, Brown WT, Li X: Expression of inflammatory cytokines, Bcl2 and cathepsin D are altered in lymphoblasts of autistic subjects. Immunobiology 2011;216:80-85.
55 El-Ansary A, Al-Daihan S, Al-Dbass A, Al-Ayadhi L: Measurement of selected ions related to oxidative stress and energy metabolism in Saudi autistic children. Clin Biochem 2010;43:63-70.

56 Sauer H, Wartenberg M, Hescheler J: Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 2001;11:173-186.
57 Lim SI, Zhong CJ: Molecularly mediated processing and assembly of nanoparticles: exploring the interparticle interactions and structures. Acc Chem Res 2009;42:798-808.
58 Van Dam PS, Van Asbeck BS, Erkelens DW, Marx JJM, Gispen WH, Bravenboer B: The role of oxidative stress in neuropathy and other diabetic complications. Diabetes Metab Rev 1995;11:181-192.
59 Wolff SP: Diabetes mellitus and free radicals. Free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. Br Med Bull 1993;49:642-652.
60 Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D: Enhanced cellular oxidative stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem 1994;269:9889-9897.
61 Maziere C, Auclair M, Rose-Robert F, Leflon P, Maziere JC: Glucose-enriched medium enhances cell-mediated low density lipoprotein peroxidation. FEBS Lett 1995;363:277-279.
62 Singha S, Bhattacharya J, Datta H, Dasgupta AK: Anti-glycation activity of gold nanoparticles. Nanomedicine 2009;5:21-29.
63 Paulmann N, Grohmann M, Voigt JP, Bert B, Vowinckel J, Bader M, Skelin M, Jevsek M, Fink H, Rupnik M, Walther DJ: Intracellular serotonin modulates insulin secretion from pancreatic beta-cells by protein serotonylation.PLoS. Biologicals 2009;7:e1000229.
64 Chandana SR, Behen ME, Juhasz C, Muzik O, Rothermel RD, Mangner TJ, Chakraborty PK, Chugani HT, Chugani DC: Significance of abnormalities in developmental trajectory and asymmetry of cortical serotonin synthesis in autism. Int J Dev Neurosci 2005;23:171-182.
65 Chugani DC: Role of altered brain serotonin mechanisms in autism. Mol Psychiatry 2002;7:S16-S17.
66 Arafah BM: Hypothalamic pituitary adrenal function during critical illness: limitations of current assessment methods. J Clin Endocrinol Metab 2006;91:3725-3745.
67 Murphy DL, Fox MA, Timpano KR, Moya PR, Ren-Patterson R, Andrews AM, Holmes A, Lesch KP, Wendland JR: How the serotonin story is being rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter gene, which functions to influence all cellular serotonin systems. Neuropharmacology 2008;55:932-960.
68 Connor EE, Mwamuka J, Gole A, Murphy CJ, Wyatt MD: Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. Small 2005;1:325-327.
69 Dani RK, Kang M, Kalita M, Smith PE, Bossmann SH, Chikan V: MspA porin-gold nanoparticle assemblies: enhanced binding through a controlled cysteine mutation. Nano Lett. 2008;8:1229-1236.
70 Fecteau S, Mottron L, Berthiaume C, Burack JA: Developmental changes of autistic symptoms. Autism 2003;7:255-268.
71 Evans JL, Goldfine ID, Maddux BA, Grodsky GM: Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? Diabetes 2003;52:1-8.
72 Robertson RP: Oxidative stress and impaired insulin secretion in type 2 diabetes. Curr Opin Pharmacol 2006;6:615-619.
73 Korshunov SS, Skulachev VP, Starkov AA: High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett. 1997;416:15-18.
74 Degirmenci I, Ustuner MC, Kalender Y, Kalender S, Gunes HV: The effects of acarbose and Rumex patientia L. On ultrastructural and biochemical changes of pancreatic b cells in streptozotocin-induced diabetic rats. J Ethnopharmacol 2005;97:555-559.
75 Tyrberg B, Andersson A, Borg LA: Species differences in susceptibility of transplanted and cultured pancreatic islets to the b-cell toxin alloxan. Gen Comp Endocrinol 2001;122, 238-251.
76 Szkudelski T: The mechanism of alloxan and streptozotocin action in beta-cells of the rat pancreas. Physiol Res 2001;50:537-546.
77 Halliwell B, Chirico S: Lipid peroxidation: its mechanism, measurement, and significance. Am J Clin Nutr 1993;57:715S-725S.
78 Chen YS, Ching Y, Liau HI, Huang GS: Assessment of the in vivo toxicity of gold nanoparticles. Nanoscale Res Lett 2009;4:858-864.