International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 3; Issue 4; October 2018; Page No. 06-10

Histopathlogical effects of gold nanoparticles on utero placenta of pregnant rat

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Abstract
Gold nanoparticles (GNPs) were widely applied in biomedicine as potential probes for imaging, drug-delivery systems and regenerative medicine. However, their applications in clinical research have raised concerns about the adverse effects of (GNPs) on human health and environment. The present study was undertaken to determine the potential histopathological effects of (AuNPs) of different size (10, 25 and 50 nm) on utero placenta of pregnant rats. Gold nanoparticles were intraperitoneally injected at 48 h post pregnancy. Four equal groups of pregnant rats were used; the first one served as a control group, the second received 75 mg/kg/day AuNPs as 10 nm in size, the third group received 75 mg/kg/day AuNPs as 25 nm in size and the forth group received 75 mg/kg/day AuNPs as 50 nm in size. All groups scarified after 15 days from gestation, utero placenta specimens were collected at the end of the experiment for histological examination. AuNPs treated group revealed wide spread histological alterations in the utero placenta structure as myometrium with marked edema with vacuolation, markedly dilated blood vessels, excess glycogen cells with cytolysis of glycogen cells and dilated fetal blood vessels with excess nucleated red cells.

Keywords: gold nanoparticles, pregnant rats, utero placenta, histopathology

1. Introduction
Nanoparticle due to enormous small size occupies a position in various fields of Nano science and nanotechnology which majorly includes biomedicine and bioscience with sub stream of therapeutic and diagnostic [1]. Many nanomaterial applications came out with high expectations from diverse fields, which were grown and diversified into medical areas, including field of clinical trials for their unique optical and physical properties [2]. Nano spheres are the particles having the size range between 10-200 nm in diameter; thwy can be amorphous or crystalline in nature and also have the ability to protect the drug from enzymatic and chemical degradation [3]. Gold nanoparticles are widely used in many fields as preferred materials for their unique optical and physical properties, such as surface plasmon oscillations for labeling, imaging, and sensing. Recently, much advancement was made in biomedical applications with better biocompatibility in disease diagnosis and therapeutics [4]. Gold nanoparticles (Au NPs) have unique physico-chemical properties, including ultra-small size from 0.8nm to 200 nm, large surface area to mass ratio, high surface reactivity, chemical inertness, biocompatibility and ease of surface fictionalization [5].
Au NPs easily penetrate the nuclear pores to influence the genetic biochemical processes and can diffuse through the mitochondrial membrane pores to alter the bioenergetics of cells that make Au NPs very helpful in intracellular targeting therapy [6]. Reduced utero placental perfusion is a likely contributing factor in pregnancy loss and pregnancy-related morbidities. Irregular uterine artery Doppler waveforms characterized by an increased pulsatility index indicative of high arterial impedance are frequently observed in pregnancies with eventual adverse outcomes such as pre-eclampsia, intrauterine growth restriction, and spontaneous pregnancy loss [7, 8]. Functional deficits in utero placental perfusion may relate to insufficient conversion of the musculoelastic spiral arterioles by invasive trophoblast cells [9]. The purpose of this study is to evaluate the histopathlogical effects of Nano gold particles on utero placenta of pregnant rats.

2. Materials and Methods
Gold nanoparticles Gold nanoparticles (10, 25 and 50 nm particle size) were obtained from (National Research Center, Cairo, Egypt).

Synthesis and characterization of gold nanospheres of 10, 25 and 50nm
Au nanosphere shapes were produced by ascorbic acid mediated reduction method. For synthesis of AuNSs with a diameter of about 25 nm (AuNS25), growth solutions of AuNSs25 (7.2 mL of 0.1 M CTAB and 0.225 mL of 0.01M $HAuCl_4 \cdot 3H_2O$) and AuNSs50 (6.4 mL of 0.1 M CTAB and 0.200 mL of 0.01 M $HAuCl_4 \cdot 3H_2O$) were separately mixed with solution of fresh ascorbic acid (0.050 mL, 0.1 M). After that, 0.1 mL and 0.026 mL of seed solution were transferred to the stirring mixtures, respectively. Finally, these solutions were stirred strongly for 10 sec until a wine-red solution was obtained; the mixtures were left undisturbed overnight.

Animals: Female Albino rats *Rattus norvegicus* (12 weeks old, 200 ± 20 gm) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (VACSERA). Animals were housed individually in clean plastic cages with steel toppings (1:1 male female ratio) with 12hrs day and

night cycle in the animal house, the temperature was ranged between 20-25˚C with a relative humidity of 55±5%. Food and water were added *ad libitum*. After one week of adaptation, female rats were mated overnight (each male rat was mated with 3 females). Day 0 of pregnancy was determined by sperm in a vaginal smear. Animals in this study were conducted in accordance with the criteria of the investigations and Ethics Committee of the Community Laws governing the use of experimental animals.

Experimental Design: Pregnant rats were randomly divided into four equal groups (10 per each) as following: the first served as control group in which pregnant rats were injected with normal saline solution intraperitoneally (ip.) 48 h of pregnancy. The treated groups; 2, 3 and 4 were ip, injected with 75 µg/kg of 10, 25 and 50nm of Au NPs; respectively, post 48h of pregnancy. All groups were scarified on day-15 of pregnancy.

Histological Studies: Tissues of utero placenta of the treated and control rats were eviscerated. Samples were fixed in 10% formalin solution, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. Several sections of 5 microns thickness were prepared; mounting and staining were carried out using Haematoxylin and Eosin for routine histological examination. Stained sections were examined using a research microscope supplied with an eyepiece micrometer according to Davenport [10].

3. Results

The histological examinations of utero placental sections of control sacrificed on 15th day of gestation are depicted in (Figs 1, 2, 3, and 4, A). The normal utero placental of female rats is constructed average myometrium consists of smooth muscle and the decidual cells develop from the endometrial cells by (decidualization) and form the basic structural matrix of the decidua, labyrinth zone and the basal zone. The basal zone is consisted of three types of differentiated cells: (1) spongiotrophoblasts, (2) trophoblastic giant cells and (3) glycogen cells; the spongiotrophoblasts are present immediately above the trophoblastic giant cell layer located at the materno-fetal placental interface. The glycogen cells form multiple small cell masses and develop into glycogen cell islands. In the labyrinth zone, there are three layers of trophoblasts, separating the maternal blood spaces from the fetal blood vessels; also blood vessels appeared in its normal structure. The outer trophectoderm, which comes into direct contact with the maternal blood, is referred to as cytotrophoblasts with a microvillous surface. Under this trophectoderm, there are two layers of syncytiotrophoblasts.

Utero placental sections of group treated with 10 nm Nano gold and sacrificed on 15th day from gestation are shown in (Figs 1, 2, 3, and 4, B). The decidualized myometrium exhibited markedly dilated blood vessels, decidua with dilated blood vessels and areas of hemorrhage, average giant cell layer, thin trophospongium with dilated blood vessels and excess glycogen cells with cytolysis of some glycogen cells and apoptotic trophoblasts. Labyrinth zone showed markedly dilated congested blood vessel, irregular maternal sinusoids and fetal blood vessels containing excess nucleated red cells and degenerated trophoblastic septa. Utero placental sections of rats treated with 25 nm Nano gold and sacrificed on 15th day from gestation are shown in (Figs 1, 2, 3, and 4, C). The myometrium revealed marked edema with vacuolation; decidua was thin in width with dilated blood vessels and areas of edema as well as average giant cell layer. Trophospongium showed average in thickness with excess glycogen with apoptotic trophoblasts, dilated congested blood vessel and labyrinth zone showed area of hemorrhage, narrow maternal sinusoids, fetal blood vessels containing few nucleated red cells and thin trophoblastic septa with giant trophoblasts. Examination of utero placental sections obtained from female rats treated with 50 nm Nano gold and sacrificed on 15th day from gestation are shown in (Figs 1, 2, 3, and 4, D). The utero placenta of this group showed marked decidualized myometrium average in thickness with markedly dilated blood vessels with vacuolation, increased mitotic figure in giant cell layer, decidua layer with dilated congested blood vessels and sever vacuolation while trophospongium average in thickness with congested blood vessels with excess glycogen cells with excess cytolysis. Also, labyrinth zone showed average in thickness, average maternal sinusoids and dilated fetal blood vessels with excess nucleated red cells and thin trophoblastic septa.

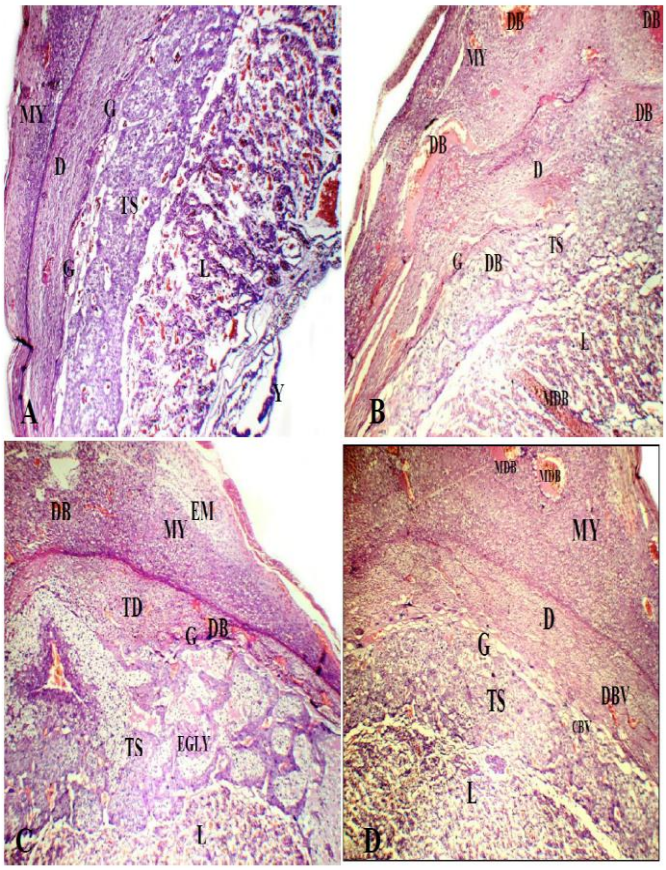

Fig 1

Fig 1: A; Transverse utero placental section in control group showing average myometrium (MY), average decidua (D), average giant cell layer (G), average trophospongium (TS), average labyrinth zone (L) and average yolk sac (Y) (H&E X

100). B; Transverse section of utero placental in group treated with 10nm Nano gold showing decidualized myometrium (MY) with dilated blood vessels (DB), decidua (D) with dilated blood vessels with areas of hemorrhage (DB), average giant cell layer (G), thin trophospongium (TS) with dilated blood vessels (DB) and labyrinth zone (L) with markedly dilated congested blood vessel (MDB) (H&E X 100). C; Transverse section of utero placental sections in group treated with 25nm Nano gold showing myometrium (MY) with dilated blood vessels (DB) and area of mild edema (EM), thin decidua (TD) with dilated congested blood vessels (DB), average giant cell layer (G), and trophospongium (TS) with excess glycogen cells (EGLY) and labyrinth zone (L) (H&E X 100). D; Transverse section of utero placental sections in group treated with 50nm Nano gold showing decidualized myometrium(MY) with markedly dilated blood vessels (MDB), decidua (D) with dilated congested blood vessels (DBV), average giant cell layer (G), average trophospongium (TS)) and labyrinth zone (L) (H&E X 100).

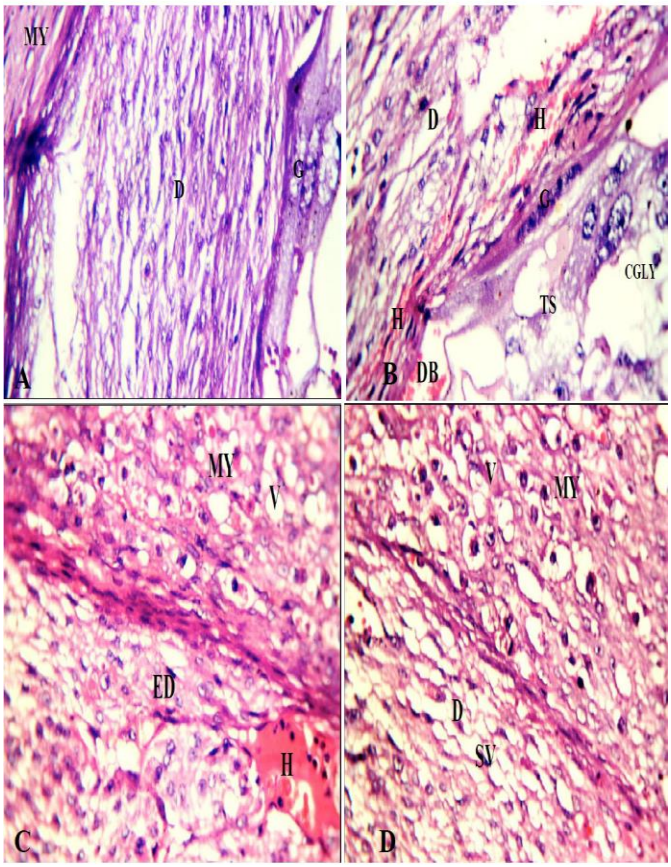

Fig 2

Fig 2: A; Transverse section of utero placental sections in control group showing average myometrium (MY), average decidua (D) and average giant cell layer (G) (H&E X 400). B; Transverse section of utero placental sections in group treated with 10nm Nano gold showing decidua (D) with areas of hemorrhage (H), average giant cell layer (G) and trophospongium (TS) showing dilated blood vessels (DB) with cytolysis of glycogen cells (CGLY) (H&E X 400). C; Transverse section of utero placental sections in group treated with 25nm Nano gold showing markedly decidualized myometrium (MY) with vacuolation (V) and marked edema of decidua (ED) with area of hemorrhage (H) (H&E X 400). D; Transverse section of utero placental sections in group treated with 50nm Nano gold showing markedly decidualized myometrium (My) with vacuolation (V) and decidua with sever vacuolation (SV) (H&E X 400).

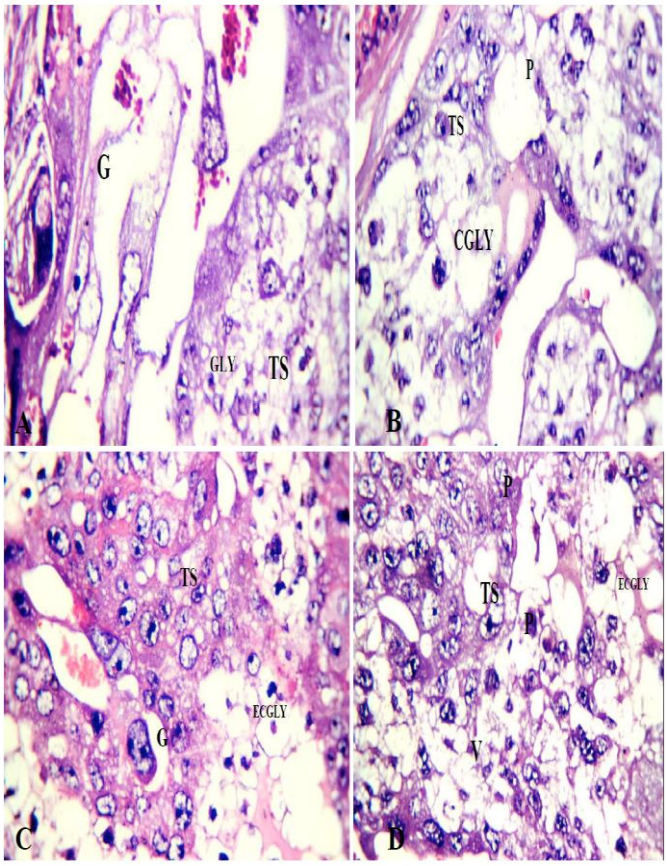

Fig 3

Fig 3: A; Transverse section of utero placental sections in control group showing average giant cell layer (G) and trophospongium showing glycogen cells (TS) (H&E X 400). B; Transverse section of utero placental sections in group treated with 10nm Nano gold showing trophospongium (TS) with apoptotic trophoblasts (P), cytolysis of glycogen cells (CGLY) (H&E X 400). C; Transverse section of utero placental sections in group treated with 25nm Nano gold showing trophospongium (TS) with excess glycogen cells showing cytolysis (ECGLY) and trophoblast giant cell (G) (H&E X 400). D; Transverse section of utero placental sections in group treated with 50nm Nano gold showing trophospongium (TS) with apoptotic trophoblasts (P) and excess glycogen cells showing cytolysis (ECGLY) and with severs vacuolation (H&E X 400).

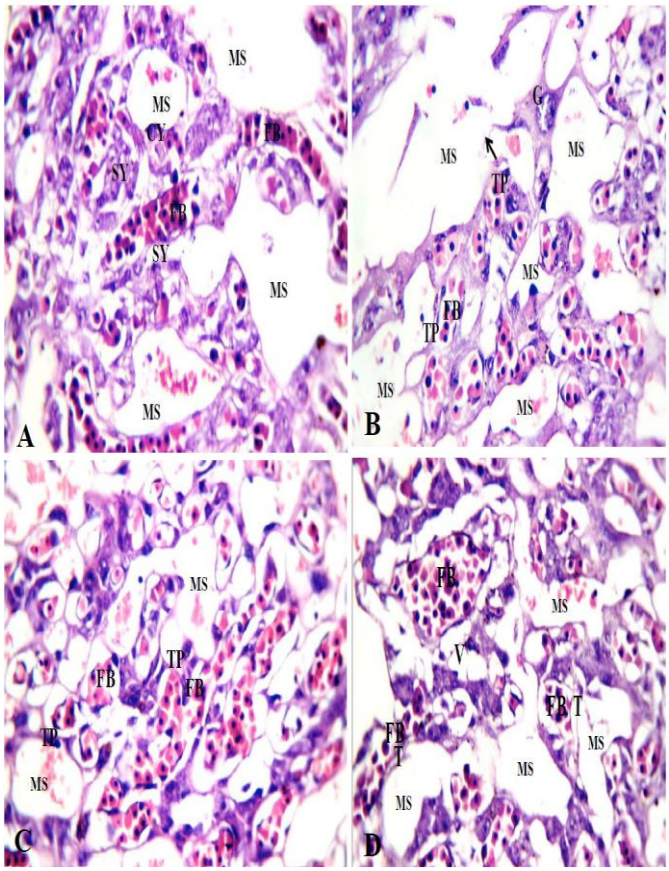

Fig 4

Fig 4: A; Transverse section of utero placental sections in control group showing labyrinth zone with average maternal sinusoids (MS) and average fetal blood vessels containing nucleated red cells (FB) with average intervening trophoblastic septa showing cytotrophoblasts (CY) and syncytiotrophoblast (SY) (H&E X 400). B; Transverse section of utero placental sections in group treated with 10nm Nano gold showing labyrinth zone showing irregular maternal sinusoids (MS), fetal blood vessels containing nucleated red cells (F) and degenerated trophoblastic septa (TP) with giant cell (G) (H&E X 400). C; Transverse section of utero placental sections in group treated with 25nm Nano gold showing labyrinth zone with average maternal sinusoids (MS), fetal blood vessels containing nucleated red cells (F) and thin trophoblastic septa (TP) (H&E X 400). D; Transverse section of uteroplacental sections in group treated with 50nm Nano gold showing labyrinth zone with average maternal sinusoids (MS), fetal blood vessels containing nucleated red cells (FB), thin trophoblastic septa (T) and vacuolation (V) (H&E X 400).

4. Discussion

The histological alterations on the placenta could by itself cause adverse effect in the embryo and inadequate embryonic nutrients may influence the morphogenesis either on its own or by modulating the homeobox genes and proto-oncogenes involved in the prenatal development [11]. In the present study, rats treated with 10 nm Nano gold and sacrificed on 15th day from gestation exhibited myometrium with markedly dilated blood vessels, decidua with dilated blood vessels and areas of hemorrhage, average giant cell layer, thin trophospongium with dilated blood vessels and excess glycogen cells with cytolysis of glycogen cells and apoptotic trophoblasts. Also, labyrinth zone showed obviously dilated congested blood vessel and irregular maternal sinusoids and fetal blood vessels containing excess nucleated red cells and degenerated trophoblastic septa. These results are in agreement with [12]; they showed that the placenta of rats treated with broncholytic drug revealed marked decrease in glycogen cell-islands in the basal zone. Glycogen cells have cystic degeneration, where abnormal retention of extened cytoplasmic vacuolation within glycogen cells. These vacuoles contain eosinophilic fibrinous material and polymorphs. The degenerated cells undergo cytolysis and subsequently coalesce into multiple large cysts that are filled with a homogeneous acidophilic mass and multiple clusters of residual glycogen cells, macrophages, erythrocytes and cell debris. Nanoparticle uptake by placenta is significantly increased at a specific size; possible transport routes for nanoparticles across the placenta include diffusion, vesicular transport, transmembranal transporters, the transtrophoblastic channel system, phagocytosis or endocytosis, or entry into fetal tissues following direct damage to placental cells [13, 14]. It is possible that Aloe barbadensis might cause cells of maternal-fetal interface (decidual cells and spongiotrophoblasts) to degenerate and die; thus stimulating trophoblasts to phagocytose and remove damaged cells. Phagocytosis acts as a biological mechanism for elimination of dead or degenerating cells. It seems likely that degeneration and finally decline of giant cells finally causes diminishment of trophoblasts [15]. The present investigation showed that; treatment of pregnant rats with 25, 50 nm Nano gold and sacrificed on 15th day from gestation, resulted in edematous myometrium with vacuolation, decidua showed thin in thickness with dilated blood vessels and hemorrhage in the labyrinth zone. In addition, narrow maternal sinusoids, fetal blood vessels containing few nucleated red cells and thin trophoblastic septa were perceptive. These results are in accordance with [16]; reported that when pregnant rats treated with antiepileptic drug; the area of trophospongium zone has been decreased in number of nucleus and cytolysis of glycogen cells. Labyrinth area showed decreased cellular strands septa had lost their cellular architecture which act as a barrier that separates the maternal blood from embryonic capillaries; resulting in admixing of maternal and fetal blood and decreased vessels formation [17]. The trophoblastic cells of the labyrinths also produced a fibrinoid substance, which appeared to be secreted into the maternal sinusoid. Fibrin could also have developed as a result of enhanced vascular leakage due to mucophylline administration. Moreover; fibrinoid accumulation in the labyrinths and the altered labyrinthine architecture, poor vascularization, would suggest a substantial reduction in placental transport functional capacity and, consequently, lead to fetal growth retardation. In the present study trophospongium zone was decreased which resulted from degeneration of trophoblasts. Also; giant cells were present that had increased vacuoles. However; giant cells act as biological elimination of degenerated trophoblasts and finally cause diminished of trophoblast cells [18, 19, 20]. The present study showed that the treatment with 10nm 25 and 20nm of AuNP has similar effect in the histological structure

of utero placenta, but any Nano particles caused metabolic alterations that might modify the exchange of substances, normally occurring between mother and fetus.

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