Original Article

Biological Impact of Gold Nanoparticle on Estradaiol and Testosterone Levels in Sera of Human Males

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Abstract:

Background: There is a little information on the potential nanoparticle risk to human health, particularly on the sex hormones.

Objectives: the present study aims to investigate the impact of gold nanoparticles potential toxicity on the levels of sex hormones testosterone (T) and estradiol (E2) in sera of human males

Patients and methods: a total of 25 healthy men volunteers aged (20-45) years participated. Four types of gold nanoparticles were used to study their impact on estradiol and testosterone levels.

Results: The results indicated inhibiting effect of gold nanoparticles on E2 level and activating effect on T level .The low impact of T/E2 by gold nanoparticles was executed at 20µl serum.

Conclusion: different types of gold nanoparticles have an impact on sex hormone binding to their receptors and thus effect on its level in the blood, hence affects the biological processes that are dependant on hormone concentration.

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Introduction:

Gold nanoparticles (GNPs) are relatively inert in biological environment, and have a number of physical properties that are suitable for several biomedical applications including bio-detection, drug delivery and diagnostic imaging (1)(2). Over the past decade with the rapid development in technologies for the chemical synthesis of GNP, a variety of particles with different sizes, shapes, structures, and optical properties are now available to researchers (3)(4). The most widely applied and simplest methods to produce GNPs use chemical reduction of gold salt to metallic gold in the presence of a capping ligand. (5)(6). GNPs have a range of properties that make them suitable for drug delivery (7). However, despite these advances toward potential therapeutic and current consumer benefit of GNPs, concern has arisen as to the possible toxicological consequences of such molecules within biologic systems and the environment (8). There is a little information on the potential nanoparticle risk to human health, particularly on their possible toxic effects on the endocrine system(9). Most toxicology data show that the surface chemistry and physical dimensions of gold nanoparticles play an important role in toxicity (10). Hormones play a key role in influencing the development of the reproductive system and subsequently in controlling its activities once developed. Testosterone is the male sex hormone synthesized in the testes, is essential for the normal development, functioning of male sex organs and the maturation of sperm (11). Estradiol in men is produced by the adrenal gland and testes. Its role is less well defined but it seems to be involved in the regulation of gonadotropins (12). The aim of present study is to investigate the impact of gold nanoparticles potential toxicity on the changes in concentrations of sex hormones testosterone (T) and estradiol (E2) in sera of male.

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Patients and methods:

The materials used in this study consisted of: 1- gold nanoparticles with three different shape gold nanosephers (GNPs 1; 30 nm, 26.79ppm) synthesized by trisodium citrate reduction or(GNPs 2; 12 nm, 46.43 ppm)synthesized by sodium borohydride reduction (13), gold nano shell (GNPs 3; 25 nm, 12.5 ppm) (14),and gold nano rod (GNP4; 10 nm, 41.04 ppm) (15). 2-Enzyme Linked Immune Sorbant Assay (ELISA) Kits for estradiol and testosterone were purchased from human (Germany) .Its principle based on the competitive binding between hormone specimen and hormone conjugated on coated well. The activation and inhibition percentages of E2 and T binding between Antigen and Antibody were calculated by comparing the hormone concentration with GNPs (10µL of GNPs was added to sample assay) and without the GNPs (10µL of deionized distilled water was added to sample assay) under the same conditions, according to the equations(16):

% activation = Activity in the presence of nanoparticles / Activity in the absence of nanoparticles x 100 – 100.

% inhibition = 100 – Activity in the presence of nanoparticles / Activity in the absence of nanoparticles x 100.

In order to study the concentration effect of GNPs on E2 or T binding with their receptors, serial concentration of GNPs were prepared by dilution stock solution with deionized distilled

water and determination of the hormones concentration under the same condition.

Sample:

A total of 25 volunteer healthy men aged (20-45) years participated in this study. Five milliliters of venous blood samples were collected, transferred into plane tube and allowed to coagulate at room temperature then centrifuged at 3000 rpm for 5 min. The resulting serum was separated and stored at (-20°C) until assay.

Statistical Analysis:

All statistical analyses in studies were performed using SPSS version 17.0 for Windows (Statistical Package for Social Science, Inc., Chicago, IL, USA). Descriptive analysis was used to show the mean and standard deviation of parameters.

Results:

Table (1) shows the mean levels and range of E2(pg/ml),T(ng/ml) and T /E2 in sera of healthy male .It is obvious from the results that they are within normal range.The activation or inhibition percentage of studied gold nano particles for E2 and T levels were studied on sera of healthy male.

Table 1: Mean levels of hormones in sera of male

Hormone	range	mean± SD
E2(pg/ml)	22.27-39.21	31.925± 7.734
T (ng/ml)	1.68-7.37	4.71± 2.619
T/E2	75.43- 187.96	147.53± 33.86

The results presented in Table (2) indicated in general inhibition effect on E2 by 4 types of GNPs while activation effect on T level was observed in presence of these GNPs.

Table 2: Activation or inhibition percentage of gold nanoparticles on E2 and T levels

Sample	E2 (pg/ml)	T (ng/ml)	T/E2	% Inhibition of E2	% Activation of T
Sera without GNPs	30.99	1.4646	4.726	------	------
Sera with gold nanosephers (GNPs1)	20.89	2.46	11.775	32.59	67.96
Sera with gold nanosephers (GNPs2)	19.038	18.32	96.22	38.56	1150
Sera with gold nanorod (GNPs3)	19.66	8.172	41.566	36.56	457.96
Sera with gold nanoshell (GNPs4)	18.6	10.539	56.66	39.98	619.58

The inhibition percentage of E2 was convergent between four GNPs types where the GNPs 1 was the most type and the GNPs4 the least type. Meanwhile the activation percentage of T by GNPs was relatively clear where the GNPs 4 was the most type and the GNPs1 the least type. The overall effect of these GNPs on both E2 and T was described in T/E2. The balance between these two hormones may be more important than their absolute quantities where it were observed that most effect was in presence of GNPs 2 and the least was in presence of GNPs1 as shown in Table 2.The different concentration of each GNPs in the present study were analysed.The results presented in figure 1 demonstrated that highest inhibition of binding E2 with its receptors was executed at 10 ppm of GNPs1, 41 ppm of GNPs 2, 20 ppm of GNPs3 and 7.5 ppm of GNPs 4. On the other hand the highest inhibition of binding T with its receptors was executed at 20 ppm of GNPs1, 41 ppm of GNPs 2, 30 ppm of GNPs3 and 12.5 ppm of GNPs 4 as shown in Figure 2. Figure 3 show the E2, T,and T/E2 levels with different antigen concentrations present in serum volume in absence and presence of GNPs1 and GNps4. The results indicated that the low impact of E2 and T by GNPs 1 and GNPs 4 was executed at 20 µl and 30 µl serum respectively. Meanwhile the low impact of T/E2 by GNPs 1 and GNPs 4 was executed at 20 µl serum.

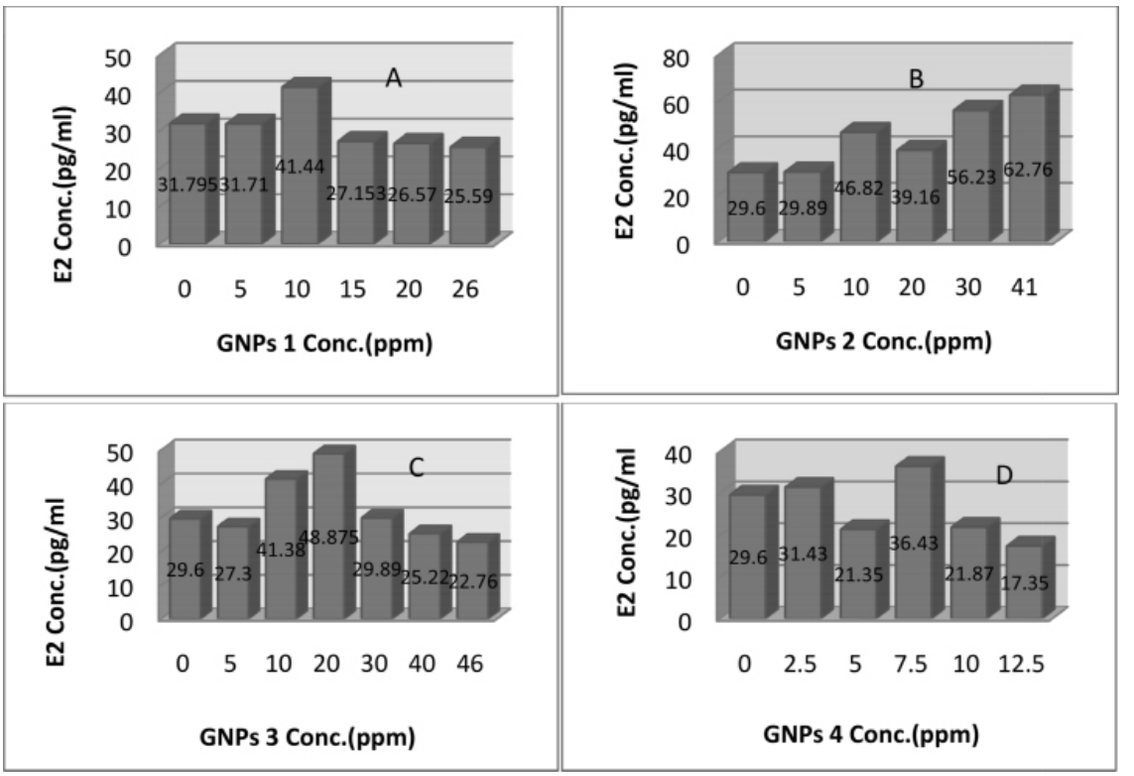

Figure1:E2 Concentration in presence of gold nanoparticles :a:GNPs 1(nanosephers 30 nm, 26.79ppm),b: GNPs 2(nanosephers 12 nm, 46.43 ppm), c:GNPs 3 (gold nanorod 10 nm, 41.04 ppm) and d: GNPs 4 (gold nanoshell 25 nm, 12.5 ppm) in different concentration.

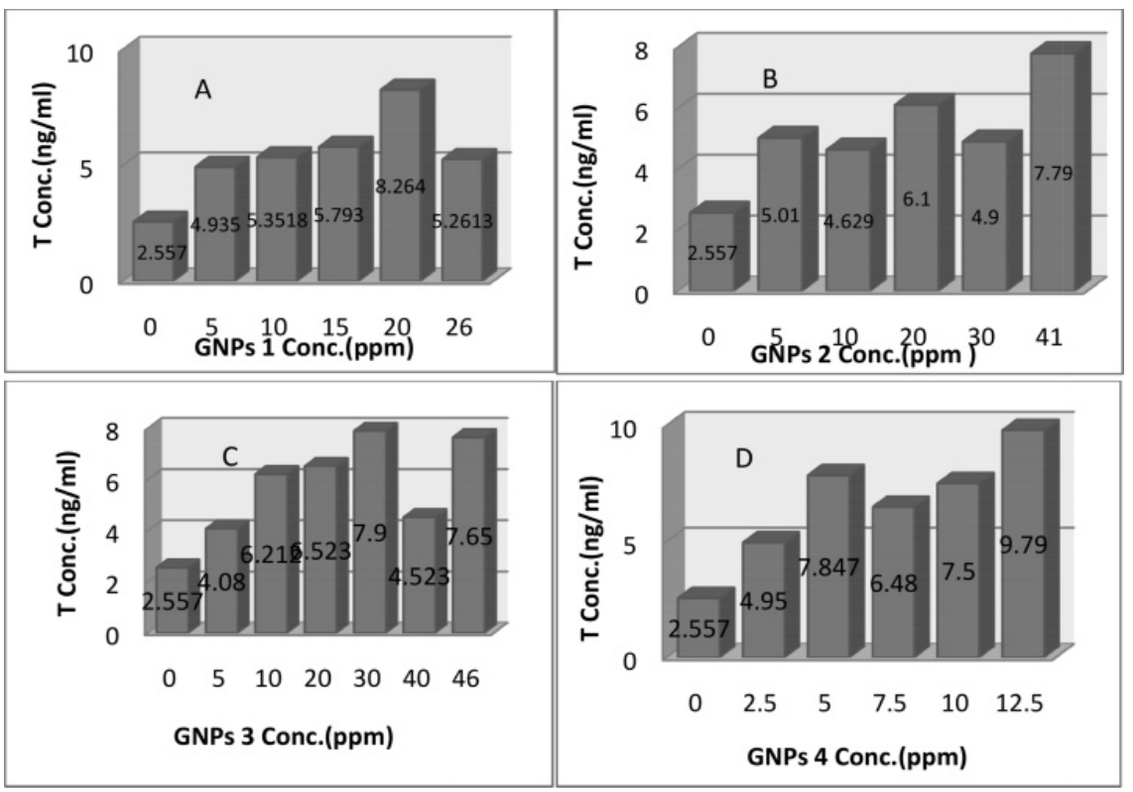

Figure 2: T concentration in presence of gold nanoparticles: a: GNPs 1(nanosephers 30 nm, 26.79ppm),b: GNPs 2(nanosephers 12 nm, 46.43 ppm), c:GNPs 3 (gold nanorod 10 nm, 41.04 ppm) and d: GNPs 4 (gold nanoshell 25 nm, 12.5 ppm) in different concentration.

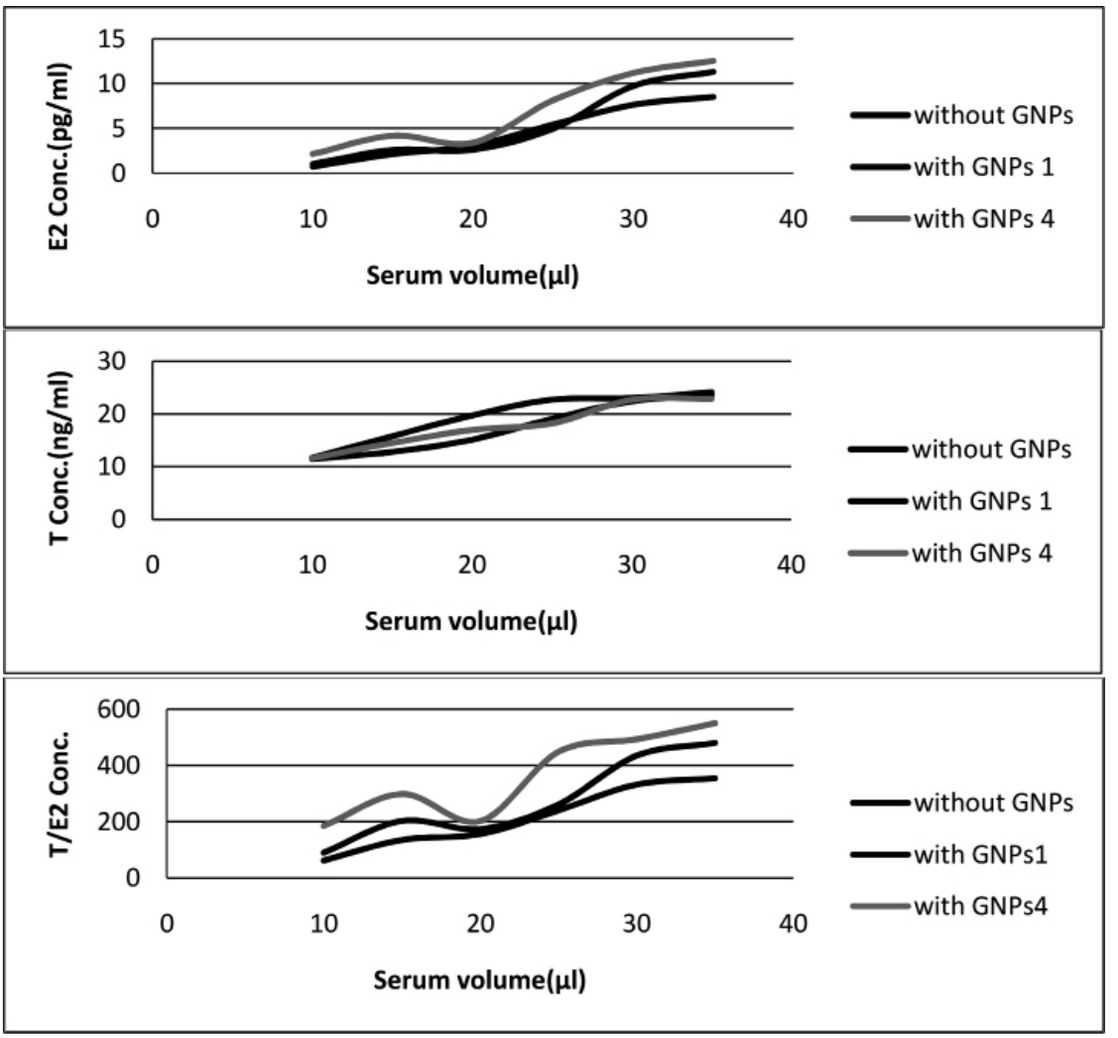

Figure 3: E2, T .and T/E2 concentrations in different serum volume without GNPs (blue line) and with GNPs1:nanosephers 30 nm, 26.79ppm (red line) and with GNPs4: gold nanoshell 25 nm, 12.5 ppm (green line).

Discussions:

To the best of our knowledge, the present study is a first one to look at the effects of nanoparticles on sex hormones binding to its receptors in human serum. Other studies focus upon nanoparticles effect on male reproductive function in Mice and cell lines. Elevated testosterone levels were indicated in previous study with a non alterations in luteinizing hormone and follicle stimulating hormone values after the administration of polyethylene glycol -NH2 capped GNP in Imprinting Control Region(ICR) mice, (17).Meanwhile Zhang XD etal. reported in their study that the toxicity of polyethylene glycol -coated gold particles is complex, and it cannot be said that the smaller particles have greater toxicity, where the toxicity of the 10 nm and 60 nm particles were obviously higher than that of the 5 nm and 30 nm particles(10). Ivo Iavicoli, etal. reported that most of the adverse effects of nanoparticles on male reproductive function are mainly due to modification of the testicular structure, impairment of spermatogenesis and alteration in the biosynthetic and catabolic pathways of testosterone(9). The chemistry surface of nanoparticles seem to be play an important role in inducing hormonal alterations (9). Larson determined in living ovarian tissue that gold nanoparticles affected production of progesterone, a sex steroid hormone that affects the production of estrogen and testosterone (18). These effects may be due to the ability of GNPs to bind to sulphur containing serum proteins as well as forming hydrophobic interactions. In this case some of the signalling proteins which interact with receptors can be bound to GNPs (19). Conner and Schmid reported in their study that protein adsorption to the nanoparticle surface can mediate the uptake of the nanomaterial via receptor-mediated endocytosis (20). Cedervall et al. demonstrated that many different plasma proteins adsorb on nanoparticles spontaneously, and that the surface chemistry of the nanoparticles in growth media/plasma is not the same as the originally synthesized materials (21).

Instead, the nanoparticles adopt the physiochemical properties of the adsorbed protein shell (22) (23). This mean that the concentration of receptor present in serum has important role in nanoparticles impact on sex hormones binding to its receptor.

Conclusion:

In conclusion; the current study found that the gold nanoparticles have impact not only on the reproductive system in terms of its production of hormones, but also has an impact on hormone binding to their receptors and thus effect on its level in the blood, which as a result affects the biological processes that are affected by hormone concentration.

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