THE EFFECT OF GOLD NANOPARTICLES ON SOME REPRODUCTIVE HORMONES IN MALE IN MALE ALBINO RATS RATTUS NORVEGICUS

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Article History: **Received**: 28.05.2022 **Revised**: 27.06.2022 **Accepted**: 27.07.2022

Abstract: Background: Gold nanoparticles[AuNPs] were highly used recently in many therapeutic approaches and medical applications, a few is known about the effects of those nanoparticles on testis histology in animal model.
Materials and methods: Thirty five healthy male adult albino rat Rattus norvegicus aged 10-12 week old with 200-230 body weight were used in the current study. Rats were randomly grouped into three groups: first group given distilled water, second group given 40mg/kg body weight of gold nanoparticles, third group given 80 mg/kg body weight of gold nanoparticles; for all the administration done orally for 60 days. After the 24 of administration animals were sacrificed and their blood was blood was withdrawed, serum obtained for the doing of hormonal assay by using ELIZA kits provided from Elabscience USA. For evaluation the level of Leutinizing hormone, Follicle Stimulating hormone, Testosteron and Estradiol.
Results: Results were explained that low doses of gold nanoparticles for 30 days have less significant than the exposure for 60 days which were indicated that there was a decrease in the levels of (T,LH, FSH.)and increased Estradol hormone causing hyper aromatase syndrome , and there were an increase in ROS levels as a result of oxidative stress.
Conclusion: Our present study indicated that a dose of 80mg\kg of body weight of gold nanoparticles could cause a deleterious toxic effects on the testicular structure more prevalently than the dose of 40 mg\kg of body weight,referring that low doses could cause less toxic effects and dose related toxicity.

Keywords: Gold nanoparticles, reproductive hormones, male, rat, estradiol**,** testosterone, LH,FSH

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DOI: 10.31838/ecb/2022.11.04.016

INTRODUCTION

Nanoparticles are becoming more common in commercially available products. Nanoparticle defined according to ASTM (American society for testing) standard from (1-100) "ASTM International , 2006 " therefore the nanotechnology can be defined as a branch of biotechnology engineering (Beer *et al*., 2012). The uses of Nanoparticles (NPs) including delivery , bio detection and diagnostic imaging for several bio medical application (Zhang , 2015 ; Barkalin *et al*., 2014; Zadeh et al.,2022) the simplest methods for produce gold nanoparticles reduction of gold salt by using chemical methods (Brust et al., 1994 ; Turkevich *et al*., 1951).There is information but a little on nanoparticle risk potential ,effect on endocrine system (Ivo *et al*., 2013; Ansari et al.,2022). physical dimensions and surface chemistry play on important role in toxicity of gold nanoparticles (zhang et al., 2011; Bokov et al.,2022) the development of reproductive system and controlling its activities by hormones play a key role such as testosterone is the male sex hormone produce in testes important for the maturation of sperm and functioning of male sex organs such as the normal development (Mader & Galliart , 2001). nanoparticles could be toxic and dangerous or can be beneficial when converted into nano (Fiorito *et al*., 2006) whit out facing any barriers the small size of nano to conquer the defens barriers of the body (Reddy *et al*.,2014; Huldani et al.,2022) .Nanoparticles can be dangerous to health for two cause : firstly, New nanoparticles are so small can penetration for cell membrane it causes toxic effects .secondly, nanoparticles very easily be absorbed by epithelial cells and skin (Mital & Manoj ,2011).using different kinds of coatings a thin layer or covering to exploit the beneficial application in biological system of nanoparticles such as dextran, albumin, polyethylene glycol , aspartic acid , etc.. using in the stability of nanoparticles in biological solution and facilitate distribution entering in cell of tissue less their toxic effect (Chen *et al*., 2006 ; Jun *et al*., 2006; Hafsan et al.,2022).
There are many studies of the endocrine disruption chemicals(EDCS) which affect the essential hormone of the male reproductive system, causes number of damaging such as testicular cancer poor semen quality, prostate diseases ,and high number or low number abnormal sperm(Knez,2013).The number leydig cell and sperm parameter indices have interruption in function of sex hormones (Baki *et al*.,2014). NPs in adult male rat showed significant reduction in LH ,FSH and testosterone (Negahdary *et al*., 2015). When exposure NPs can cause alteration or modulation in the cell ,in the cell signalling pathways processes NPs can cause alteration or modulation .Nps act as EDCS, cause hormonal disruption, impact fertility, EDCS disrupt the bodies, normal function(Dagar and Bagchi 2020).Au NPs decrease testosterone production in leyding cell when accumulate in

testes by decrease the expression 17-α hydroxylase NPs can diminish affecting the quality sperm(Liu *et al.*, 2020)

MATERIALS AND METHOD

Gold nanoparticles ,Au NPs (VCN 4021 w ,Iran), which used in the current study was have the following characteristic :

- appearance: color red solution
- weight: concentration 100 ppm
- additives :Au, morphology : spherical
- size range :5-20nm
- product number: VCN 4021 w

Figure 1.

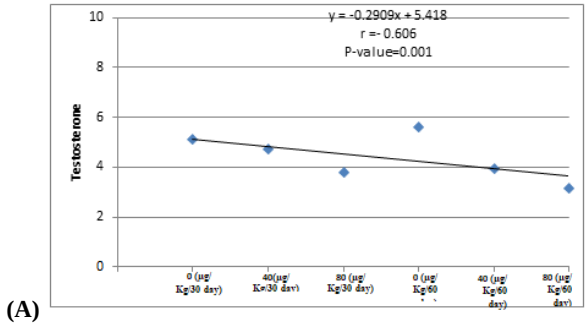
Thirty five healthy male adult albino rat rattus norvegicus aged 10-12 week old with 200-230 body weight were used in the current study.Animals were housed in the animal house of Babylon university, and left a week for acclimatization with giving food and water ad libitum with half to half nigh and light period. Rats were randomly grouped into three groups :first group given distilled water, second group given 40mg/kg body weight of gold nanoparticles, third group given 80 mg/kg body weight of gold nanoparticles; for all the administration done orally for 30 days and 60 days. After the 24 of last administration, blood was withdrawal, serum obtained for the doing of hormonal assay by using ELIZA kits provided from Elabscience USA. For evaluation the level of luteinizing hormone, Follicle stimulating hormone, Testosterone

Data analyses:

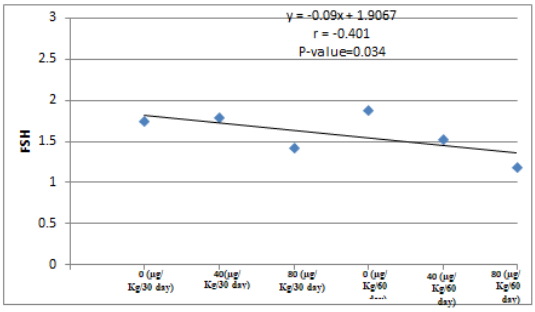
The data analysed using SPSS(version 20,SPSS Inc. checago).all data was presented as mean and standard Error(SE)statistical level of significance was set at P<0.05 and the variable were analysed by(ANOVA) by one way analysis ,the difference is less significant to use LSD,(Griffith,2007).

RESULT AND DISCUSSION

Physiological studies:



(A)



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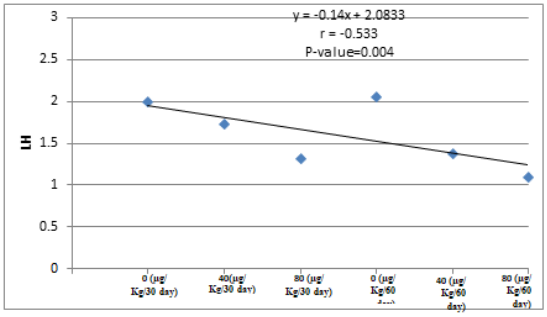

(C)

Parameters	Period (day)	Dose(µg/Kg) 0 (control)	40	80
		Mean±S.D		
Testosterone	30	5.11±0.24 c	4.74±0.5 b	3.78±0.4a
	60	5.64±0.6 c	3.96±0.1 b	3.17±0.7a
p-value			0.003**	0.043*
FSH	30	1.75±0.16 a	1.79±0.3a	1.42±0.4a
	60	1.88±0.40 c	1.52±0.3b	1.19±0.3a
p-value			0.191	0.240
LH	30	2.00±0.25b	1.73±0.6ab	1.32±0.4a
	60	2.05±0.6b	1.37±0.2a	1.09±0.1a
p-value			0.152	0.212
Estradiol	30	52.11±5.7a	61.59±3.2b	103.99±12.2c
	60	53.73±4.9a	118.63±12.1b	119.39±26.3b
p-value			0.024*	0.186
TAO	30	1121. ±22.10b	1110.99±65.4b	1012.71±73.0a
	60	1126.76±58.4c	975.67±8.7b	852.74±85.4a
p-value			0.007**	0.003**

(d)

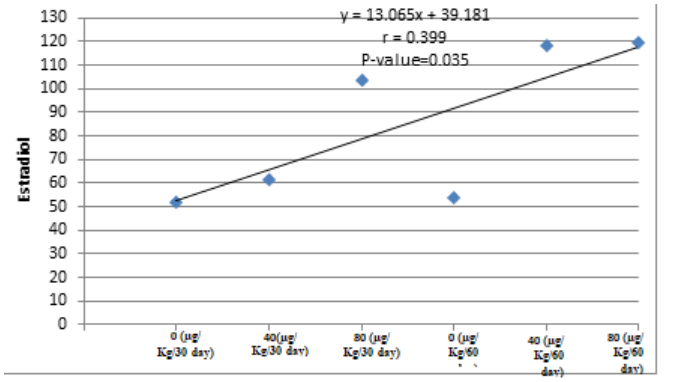

Figure 2.

Effect of Au NPs at a Concentration of 40µg/kg ,80µg/kg on Some Reproductive Hormone Levels such as Testosterone, LH,FSH and Estradiol for 30 days:

Our result showed a slight significant difference at the 40µg/kg ,80µg/kg compared to the control group, that different doses of gold particles have an effect on testosterone secretion in the testes. The aim of the study is to know the toxicity resulting from nano-gold according to different doses and duration of exposure. This hormonal disorder resulting from the treatment is due to the negative effect on leydig cells (interstitial cells) , whereby the activity of mitochondria decreases and this consistent with Carlson *et al*.,(2008), and Yan *et al*.,(2016) who were reported similar observation ,due to the high free radicals reactive oxygen species ROS which works on oxidation of molecules(proteins) and cyclooxygenase . And the decrease antioxidant enzyme causes the onset of oxidative stress on polyunsaturated n fatty acids, which make up a high percentage of tissue cells as a result of being affected by reactive oxygen species. Causes degeneration of leydig cells and low testosterone hormone, responsible for perpetuating the work and functions of the reproductive system of the testes, epididymis and gonads(Sikka *et al*.,1995; Sharma &Agarwal,1996). In another study showed Omar & Kamar (2021) that a concentration 40µg/kg of Au NPs able to induce apoptosis in the hypothalamus and secretory cells resulting from accumulation of Au NPs , where there is significant increase in expression of Bax and Caspase3 which is an intrinsic pathway for apoptosis, thus reducing the hormone FSH, LH. Our result also show that there is a significant increase in the hormone estradiol as a result of injections with different doses and at spaced intervals .Estradiol is the predominant form of estrogen that plays an important role in the reproductive sexual function of male, with the aromatase enzyme that converts testosterone into estradiol in the presence of estrogen receptors in the brain, penis, and testicles . Estradiol creates the highest level in the brain associated with sexual arousal and aromatase this agree with(Savic *et al* 2005) . It has been show that there is a significant difference between low testosterone and high estradiol, causing infertility and weak sexual desire due to the accumulated doses of gold particles and a hormonal disorder occure in the pituitary gland or hypothalamus as a result of oxidative stress and formation of ROS this corresponds to(Mancini *et al*., 2005) .where there is an indispensable hormonal interaction highly regulated by estrogen in male .

Effect of AuNPs at a Concentration of 40µg /kg, 80µg/kg on Some Reproductive Hormones Levels such as Testosterone ,LH,FSH and Estradiol for 60 day:

The result showed a significa nt decrease ($p< 0.05$) in sex hormone according to duration of exposure to gold nanoparticles, which is the most common mechanism behind the reproductive toxicity of NPs, this corresponds with Morgan *et al*.,(2017) which showed thatTiO2 nanoparticles have a toxic effect on the male reproductive system of rats, depending on the exposure time ,and this agree with(Li *et al*.,2009). Gold nanoparticles can pass through the blood- testis barrier and accumulate in the genitals damaging leydig cells , sertoli and germ cells causing dysfunction disruption of the levels of secreted hormones such as a result of oxidative stress, inflammation and apoptosis (Wang *et al*., 2018; Hussein *et al*.,2016) .

Another hypothesis is that the hormonal imbalance due to the gold particles reduces the gene expression of the protein (star) and prevents the transfer of cholesterol in the mitochondria to inner membrane and thus prevents the conversion of cholesterol to pregnenolone and reduce the level of T hormone , where LH binds to its receptors on leydig cells to produce pregnenolone by gene expression of The protein(star) is an acute regulatory protein steroidal origin and this is consistent with Waterman and Keeney (1992). Also in agreement with Liu *et al*.,(2020) who suggested that gold particles reduce T hormone production in leydig cells by inhibiting the gene expression of 17 α-hydroxylase after repeated administration, an enzyme important in androgen synthesis. Higher doses of Au NPs cause a significant decrease in the values of LH,FSH as a result of pituitary gland disturbance due to increased oxidative stress and an increase in reactive oxygen species and this is consistent with (Mc Lachlan *et al*.,2002) and the reduction of antioxidant that lead to oxidation of lipids in cell membranes including the brain. Where Knol showed that oxidative stress of all kinds leads to activation of the hypothalamic-pituitary-adrenocortical axis which leads to inhibition hypothalamus – pituitary –testis axis and hormone secretion CRH corticotropin releasing hormone inhibits GnRH hormone and latter inhibits and reduces the hormone FSH,LH from the pituitary gland then decrease in level of testosterone which effect on process of spermatogenesis .We also notice a significant increase in estradoil hormone during the 60 day dosing, considering that all cells that participate in the production of sperm contain aromatase and estradiol receptors because of the important

role of estradiole in formation of sperm,as the source of estrogen in adult testes in leydig cell and immature sertoli cell . as a result of repeated administration of Au NPs, it to increase in estraoiol as a result of hormonal imbalances, which led to the possibility of developing hyper- aromatase syndrome as a result of genetic mutations, affecting the gene encoding the aromatase enzyme(Yauk *et al*.,2008).Or cerebral hypogonadism may occur as a result of hypothalamic or gonadotropic disorders as a result of nano toxicity ,it is pathological condition in which the body does not produce enough testosterone and an increase in the production of estradiol which play a role in the development of gonado and the formation of sperm this compatible with(Dadhich *et al*.,2017 ;Rastrelli *et al*.,2018) ,there is evidence to suggest that estrogen affects the gonadotropins and then inhibits LH on the leydig cells thus affecting T and sperm reduction this is consistent with(cigorrage *et al*., 1980;Atanssova *et al*.,1999) and Newsholme and Leech,(2010) show that estradiol appears to be involved in regulating Gonadotropin . Another assumption is that Au NPs have an effect not only on the reproductive system in terms of hormone production, but also on binding of hormones to their receptors thus affecting the biology of hormone concentration ,as nano materials adopt protein adsorption on surface of the Au NPs this agree with(Lynch *et al*.,2007 ;Lynch *et al*., 2008) it mean the concentra tion of the receptor has a necessary role in the effect on the gold particles on hormones associated with the receptor. Because the mechanism of endocrine hormones does not work directly, but rather combines with receptors which are large protein substances located on the cell membrane ,cell cytoplasm, or the nucleus which contains most of the receptors for steroid hormones (Dahlman- wright *et al*., 2006).

CONCLUSION

This study reached the following conclusion: Gold nanoparticles reduce hormones(T,LH, FSH.)and increased Estradol hormone causing hyper aromatase syndrome .The harmful effects depend on the concentration as the concentration increases the toxic effect increases, also the surface area increases as the diameter of the particles decreases, duration of exposure and method of administration. Gold nanoparticles have ability to penetrate the brain barrier and cause hormonal disorder. due to oxidative stress and ROS formation and affect the leydig and sertoli cells according to duration of exposure.

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