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## The ability of green silver nanoparticles to prevent negative effects of X-ray on some physiological parameters in albino male rats

Samera H. Abdullah

Nursing Department, Instituted Kirkuk Technical, North Technical University, Kirkuk, Iraq

### Abstract

The present study was used 20 adult male rats that distributed to four groups (each group consist 5 rats); control group that administrated normal saline, rats group that exposure to X ray for two weeks, rats group that exposure to X ray with 50ug of green silver nanoparticles for two weeks, rats group that exposure to X ray with 100ug of green silver nanoparticles for two weeks. The results show high significant increased ( $P < 0.01$ ) in levels of malonedialdehyied (MDA), and high significant decreased ( $P < 0.01$ ) in levels glutathione (GSH) and significant increased ( $P < 0.01$ ) in levels of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), compared with control group. Histological section of liver sections that prepared from rats group that exposure to X ray show lymphocytes infiltration, thickening wall and congestion of central veins. when used green silver nanoparticles with X ray, the results showed non-significant changes ( $P < 0.01$ ) in all parameters compared with control group. It was concluded that green silver nanoparticles has been potential role against the negative effects of X ray in adult male rats.

**Keywords:** green silver nanoparticles; X-ray; liver functions.

## قابلية الجزيئات النانوية الخضراء للفضة على منع التأثيرات السلبية للإشعاع السينية لبعض المعايير الفسلجية في ذكور الجرذان البيض

سميرة حسن عبدالله

المعهد التقني كركوك، قسم تقنيات التمريض، الجامعة التقنية الشمالية، كركوك، العراق

### الخلاصة

استخدمت الدراسة 20 ذكر جرذ بالغ وقسمت الى اربع مجاميع (كل مجموعة تضم 5 جرذان): جرعت مجموعة السيطرة بالمحلول الملحي الفسلجي، مجموعة الجرذان المعرضة للإشعاع السينية لمدة اسبوعين، مجموعة الجرذان المعرضة للإشعاع السينية ومجرعة بـ 50ug من محلول الجزيئات النانوية الخضراء للفضة لمدة اسبوعين، مجموعة الجرذان المعرضة للإشعاع السينية ومجرعة محلول بـ 100ug من الجزيئات النانوية الخضراء للفضة لمدة اسبوعين. اظهرت النتائج زيادة عالية المعنوية ( $P < 0.01$ ) في مستويات MDA مع انخفاض عالي المعنوي ( $P < 0.01$ ) في مستويات الكلوتاثيون وزيادة عالية المعنوية ( $P < 0.01$ ) في مستويات Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), و Alkaline Phosphatase (ALP) في المجموعة المعرضة للإشعاع السينية مقارنة مع مجموعة السيطرة ومن الناحية النسجية اظهرت مقاطع الكبد المحضرة من مجموعة الجرذان المعرضة للإشعاع ارتشاح الخلايا

اللمفية مع تثخن جدران واحتقان الاوعية المركزية. لكن بعد استخدام الجزيئات النانوية الخضراء للفضة اظهرت النتائج عدم وجود اي تغيرات معنوية ( $P < 0.05$ ) في معايير مقارنة مع مجموعة السيطرة. استنتجت الدراسة أن الجسيمات النانوية للفضة الخضراء تلعب دوراً محتملاً ضد التأثيرات السلبية للأشعة السينية في ذكور الجرذان البيض البالغة.

## Introduction

silver nanoparticles (AgNPs) has been used in commercial products such as medical products, household and personal care, also in textiles, with food products [1]. Biological synthesis of AgNPs could has been implementation in the field of medicine particularly as drug carrier, diagnosis purposes, anti-carcinogenic effect, antibacterial, antifungal [2], antiviral [3] anti-inflammatory and antioxidant effects [4]. The development of biosynthetic methods to obtain silver nanoparticles based on silver ions and natural plant extracts (Rich in reducing, capping, and stabilizing agents) radically changes the perspective on its adverse effects, since this green synthesis method allows obtaining silver nanoparticles with more biocompatibility [5-6]. *Trigonella foenum-graecum* commonly known as Fenugreek in England, in Japan koroha, in India Methi and in China Ku. Tou, Fenugreek goes to the family of Fabaceae [7]. The seeds of the Fenugreek possess toxic oils ,volatile oils and alkaloids have been shown to be toxic to parasites, bacteria and fungi [8]. On other hand, fenugreek seeds used as cancer therapy in china medicine. The seeds also contains some active sex substances like trimethyl amine [9]. Fenugreek contains saponins, flavor, hemicelluloses mucilage, trimethyl amine, tannins and pectin [10-11]. So, the aim of present study is to the ability of silver nanoparticles to prevent negative effects of X-ray.

## Materials & methods

### Animal model

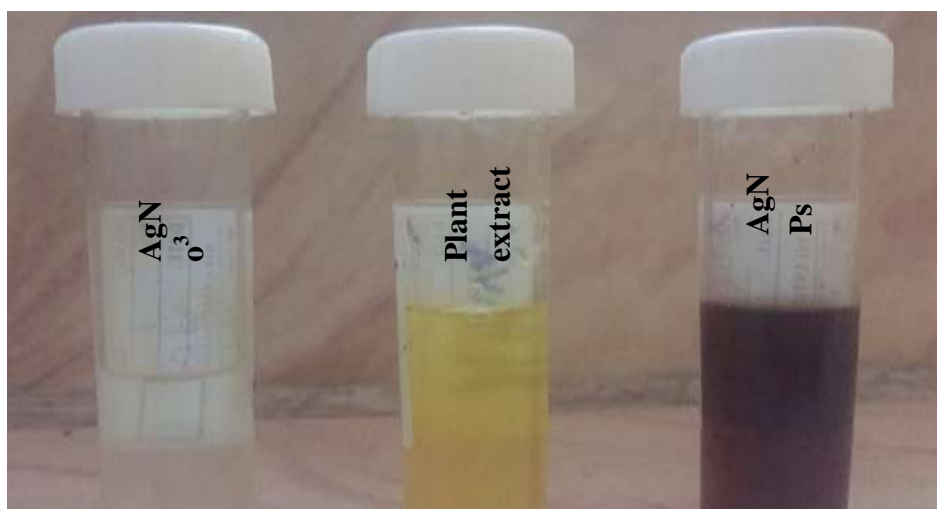
In the present study twenty adult male rats, (wt 225-250 mg with age6-10 Mon) obtained from Veterinary college/ Kirkuk University, and kept in standard environmental conditions from pellet diet and water.

### *Trigonella foenum- graecum* L. extraction

*Trigonella foenum- graecum* L. dried seeds were grounded by using an electrical grinder. The powder defatted by soxhelt apparatus, then it was sequentially extracted by adding 140 gm from powder of seeds. After that, the mixture (powder and ethanol) filtered. The filtrate was evaporated by rotary evaporator vacuu. Alcohol powder extract was placed in dark bottle and stored at 8°C for further use [12].

### Synthesis of Silver nanoparticles

18 ml of 2mM of silver nitrate solution was prepared. 2 ml of peel extract was added to the solution of silver nitrate. AgNO<sub>3</sub> ions bioreduction occurred within 5hrs. Peel extract is yellow color which converted to the brown color that indicate the formation of silver nanoparticles [13], as show in Figure-1.



**Figure1-synthesis of green silver nanoparticles**

### Experimental design

Twenty adult male rats are exposed X-ray at 70 kvp and at 0.32 second/day for two week and divided as follow (each group consist five rats):

- I. Control group were received normal diet only for two weeks and then killed.
- II. Second group were exposed to X-ray for two weeks, and then killed.
- III. Third group exposed to X-ray for two week and administrated with of 50ug/kg AgNPs at same time, and then killed.
- IV. Fourth group were exposed to X-ray for two week and administrated with 100ug/kg AgNPs at same time, and then killed.

### Prepare of blood solution

Blood samples were collecting from rats heart puncher under anesthesia, and put in test tubes (tubes without any anticoagulant factor). Then, the tubes were centrifuge for 10 min to obtain on the serum that stored in deep freeze until used

### Serological tests

the level of serum MDA was determined by a modified procedure using the thiobarbituric acid reaction substance (TBARS) methods [14]. Serum glutathione (GSH), on the other hand, was determined by a modified procedure utilizing Ellman's reagent [15]. ALT, AST and ALP level was measured using the method described by [16].

### Statistical analysis

The Data were analyzed using a statistical Minitab program. A statistical difference between the means of the experimental groups was analyzed using one way analysis of variance (ANOVA).

### Results

#### Liver function tests

AST ( $98.42 \pm 6.86$ ), ALT ( $126.72 \pm 16.64$ ) and ALP ( $184.51 \pm 13.21$ ) in rats exposure to X rays show high significant increased ( $P < 0.05$ ) compared with control rats ( $21.42 \pm 3.43$ ,  $18.54 \pm 7.43$  and  $53.38 \pm 4.34$  respectively). AST ( $38.83 \pm 5.39$ ), ALT ( $38.34 \pm 6.54$ ) and ALP ( $74.21 \pm 5.78$ ) in rats exposure to X rays and administrated with 50ug AgNPs show significant increased ( $P < 0.05$ ) compared with control rats. While, AST, ALT and ALP in rats exposure to X rays and administrated with 100ug AgNPs show no significant changes ( $P < 0.05$ ) compared with control rats as shown in Table-(1).

**Table 1-The levels of AST, ALT and ALP in serum of the groups**

Parameters Groups	AST (mg/dl)	ALT (mg/dl)	ALP (mg/dl)
I	$21.42 \pm 3.43$ c	$18.54 \pm 7.43$ a	$53.38 \pm 4.34$ c
III	$98.42 \pm 6.86$ a	$126.72 \pm 16.64$ c	$184.51 \pm 13.21$ a
III	$40.83 \pm 5.39$ b	$38.34 \pm 6.54$ b	$74.21 \pm 5.78$ b
IV	$20.65 \pm 4.27$ c	$20.51 \pm 3.56$ a	$49.39 \pm 8.72$ c

Note: same letters mean non-significant changes and different letters mean significant changes.

#### MDA and GSH

MDA ( $2.87 \pm 0.32$ ) and GSH ( $0.39 \pm 0.072$ ) in rats exposure to X rays show high significant increased (MDA) and decreased (GSH) ( $P < 0.05$ ) compared with control rats ( $1.63 \pm 0.12$  and  $0.568 \pm 0.011$  respectively). MDA ( $1.92 \pm 0.09$ ) and GSH ( $0.443 \pm 0.049$ ) in rats exposure to X rays and administrated with 50ug AgNPs show high significant increased ( $P < 0.05$ ) compared with control rats. While, The count of MDA and GSH in rats exposure to X rays and administrated with 100ug AgNPs show no significant changes ( $P < 0.05$ ) compared with control rats as shown in Table-(2).

**Table 2-**The levels of MDA and GSH in serum of the groups

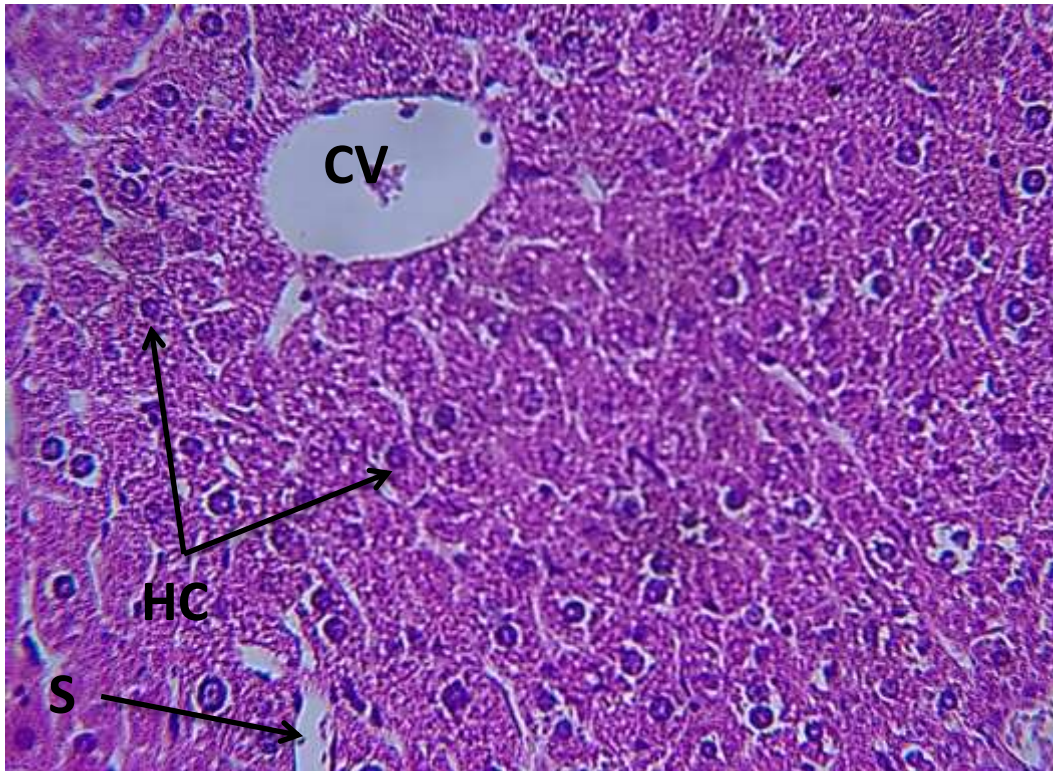
Parameters Groups	MDA (mmol/l)	GSH (mol/l)
I	1.63 ± 0.12 c	0.568 ± 0.011 a
III	2.87 ± 0.32 a	0.39 ± 0.072 c
III	1.92 ± 0.09 b	0.443 ± 0.049 b
IV	1.58 ± 0.12 c	0.561 ± 0.045 a

### Histological study

The cross section of control group liver show normal form and shape of central vein and the hepatocytes that arrangement as radical row with normal sinusoids Figure-(2), in rats liver that exposure to X rays show thickening wall of central vein with lymphocytes infiltration and congestion of blood vessels Figure-(3), in rats exposure to X rays and administrated with 50ug AgNPs show normal central vein and the hepatocytes in most regions but some hepatocytes appear degenerative changes Figure-(4), in rats exposure to X rays and administrated with 100ug AgNPs show normal central vein and the hepatocytes that arrangement as radical row with normal sinusoids Figure-(5).

### Discussion

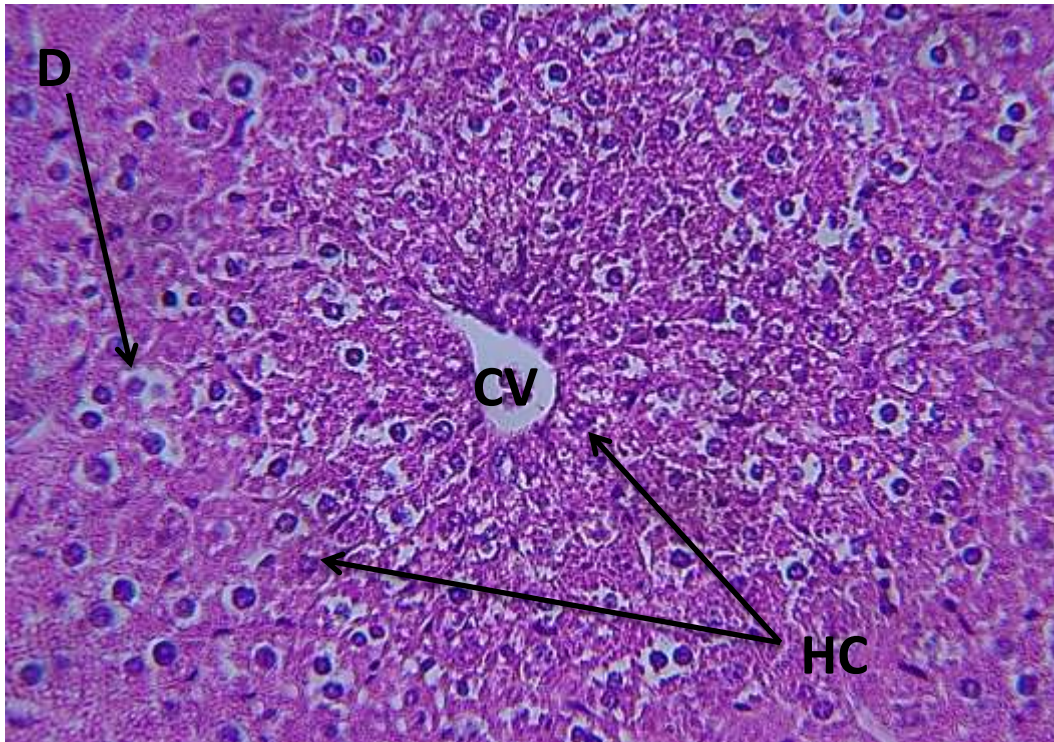
The present study showed increased in ALT, AST and ALP levels in rats exposure to X rays, the increased in levels of ALT, AST and ALP mean that liver has different lesions which lead to increase in levels of these enzymes [17], this study agreement with Ismail and Huseyin (2008) who referred that exposure to X rays lead to ALT and AST, they suggest that the X rays lead to form free radical that causes different lesions in tissues [18]. The results show increased MDA and decreased GSH levels that is in agreement with Ray et al. (2000) who referred that the exposure to ionic ray lead to increased levels of MDA and suggest that the ionic ray induced to free radical that attack the cells and lead to oxidative effects which causes degenerative changes [19]. Radiation produces reactive oxygen species, which leads to lipid peroxidation, protein oxidation and DNA damage [20]. High MDA levels may be indicative of oxidant damage to the mitochondria and myocyte membranes that could promote cell death due to membrane damage termed as radiation induced apoptosis [21]. On other hand, histological sections of X-ray group show infiltration of lymphocytes with thickening wall of central vein and congestion some of them that is in agreement with Jameel et al. (2015) who referred the exposur to X-ray lead to different lesions in rat liver including infiltration of lymphocytes and congestion of central veins. They suggest that the X-ray lead to increase the free radical that destroyed the membrane of hepatocytes [22]. The using green silver nanoparticles to protect against the negative effects of X-ray show a potential role where this ability of silver as drug and different materials delivery vehicles [23]. Also, the green silver nanoparticles have been antioxidant properties by scavengers the free radicals produced by radiation.



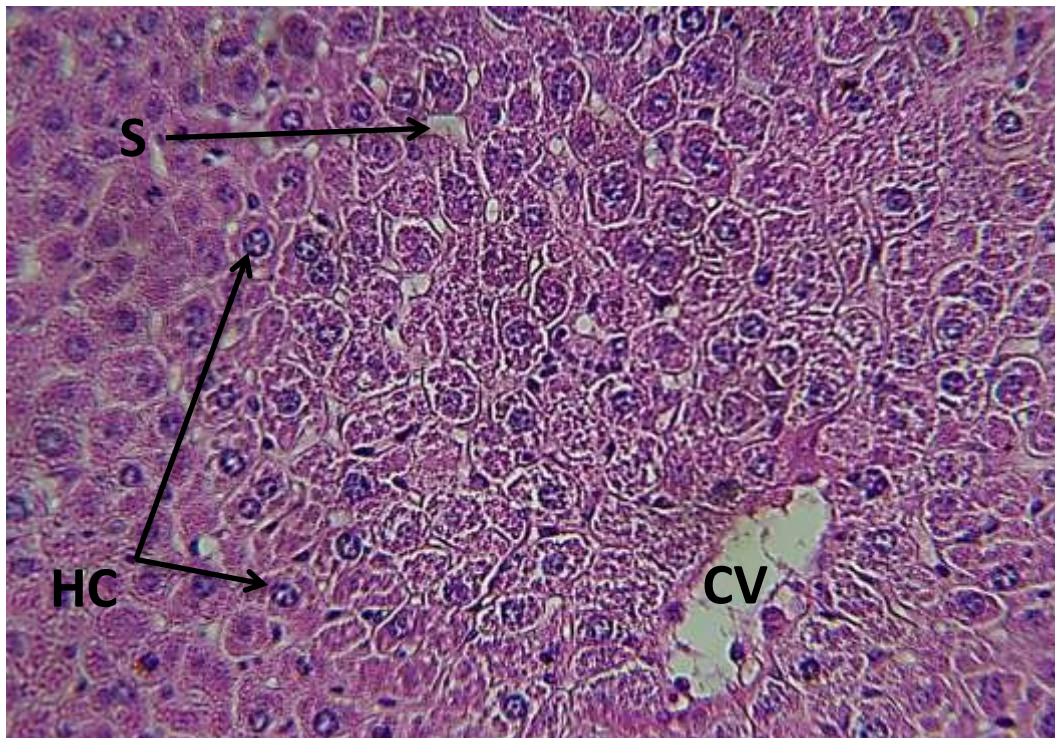
**Figure 2-**liver of control group show normal central vein (CV), hepatocytes (HC) and sinusoids (S) 400X H&E.



**Figure 3-** liver of X-ray group show thickening wall of central vein (TW), congestion (CON) and lymphocytes infiltration (IL) 400X H&E.



**Figure 4-**liver of X-ray and 50mg/kg AgNPs group show central vein (CV), hepatocytes (HC) with degeneration (D) some of them 400X H&E.



**Figure 5-** liver of X-ray and 100mg/kg AgNPs group show central vein (CV), hepatocytes (HC) and sinusoids (S) 400X H&E.

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