Iraqi Journal of Cancer and Medical Genetics

Green Synthesized Silver Nanoparticles using Crocus sativus L Extract after reduces Prehepatocellular Carcinoma In Rats

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Abstract:

In this study, the effects of green synthesized silver nanoparticles (Ag NPs) using crocus sativus L were investigated on the liver tissues of white albino rats after induced the prehepatocellular carcinoma using diethylnitrosamine. Thirty male albino rats weight (150-200) gm ,were used by dividing them into five groups, each group contains 6 rats. Group 1(control group) was given food and water like other groups by liberty. Group 2 was intraperitoneal injected with single dose of diethylnitrosamine200 mg/kg b. wt . Group 3 was intraperitoneal injected with a AgNPs 200 mg/kg b. wt for six weeks . Group 4 was intraperitoneal injected with single dose of diethylnitrosamine 200 mg/kg b. wt followed by, intraperitoneal injected with a AgNPs 200 mg/kg b. wt for six weeks . Group 5 was intraperitoneally injected with a AgNPs 200 mg/kg b. wt for six weeks ,followed by intraperitoneal injected with single dose of Diethylnitrosamine200 mg/kg b. wt . All animals were sacrified at the end of experiment. The histophathological studies revealed that group 1 have normal hepatocyte. Group2 have sever necrosis, fatty change , atypical cells and bile duct proliferation . Group 3, have normal hepatocyte cell with mild necrosis. Groups 4, have certain area of necrosis , inflammatory cells infiltration ,congestion and mild fatty change, no atypical cells were seen. Group 5 the liver section showing certain area of necrosis , inflammatory cells infiltration ,mild fatty change and no atypical cells.

Key words: *Nanoparticles, Silver nanoparticles, Albino male rat, prehepatocellular carcinoma.*

Introduction:

Nanotechnology known as the formation, utilization and installation of materials at a scale up to 100 nm in diameter (1). It was noted that physical and chemical properties change when decrease the particle size to nonoscale (2). Their characteristics based on specific features such as size, distribution, morphology (3) and high surface /volume ratio(4).

Nanobiotechnology, an important branch of nanotechnology, it is the using of green methods for the synthesis of nanoparticles, which involve clean nontoxic chemicals, ecofriendly solvents and renewable materials ,it is alternative to the conventional physical and chemical methods (5). Silver nanoparticles (AgNPs) are the most quickly rising classes of nanoparticles which are the noble metal nanoparticles that have being used for studying extensively due to its various biological properties (6). Biological synthesis of AgNPs could have been implementation in the field of medicine particularly as anti-carcinogenic effect, drug carrier, diagnosis purposes, antibacterial, antifungal (7), antiviral (8) antioxidant and anti-inflammatory effects (9).

Hepatocellular carcinoma (HCC) is a highly malignant disease with poor prognosis, its account for 80% to90% of primary liver cancer The rate of HCC in male are 2 to 4 times higher than in females (10). The major risk factors for HCC worldwide are chronic infection with hepatitis B and C, alcoholic and metabolic liver diseases(11).Other factors obesity, environmental pollutants, aflatoxin exposure(12), and nitrosamine consumption (13).

Diethylnitrosamine (DEN) is a potent hepatocarcinogen, produces primary metabolic activation resulting in initiation of liver carcinogenesis (14). It is used to study the effects of many drugs and treatment on hepatocellular carcinoma(HCC) (15). It is metabolized by P450 cytochrome enzyme to form unstable metabolites that react with DNA of cells results in mutation, forming promutagenic adducts leading to HCC

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(16). In the current study, such an approach is used to assess the potential effects of AgNPs on the liver tissues after induce prehepatocellular carcinoma by using diethylnitrosamine.

Materials and Methods:

Diethylnitrosamine was obtained from Sigma Aldrich. AgNPs was synthesized using a green bio synthesis method by reducing AgNO3 solution with aqueous extract of crocus sativus L according to Thamer etal,2014.

Experimental Animals

Adult male albino rats with body weight of (150-200)gram ,were obtained from Iraqi center of cancer and medical genetic research, were housed in plastic cages under controlled environmental conditions (24°C and a 12 h light/dark cycle) one week before starting the experiment as acclimatization period. The animals were fed with a standard diet and provided with drinking water and libitum.

Experimental design

30 adult male albino rats were divided into five groups with 6 animals in each group.

Group 1 (Control): Animals were injected intraperitoneal with 1 ml saline single dose.

Group 2 (HCC - Induced Untreated) (Positive control): Animals were induced for pre- HCC by a single intraperitoneal injection of DEN 200mg/kg body weight, dissolved in 25 ml normal saline(18) . After 2-week recovery period, the promoter carbon tetrachloride (CCl4) (3ml/ kg body weight) single dose weekly subcutaneous injection for 6 weeks(19).

Group 3 : Animals were injected intraperitoneally AgNPs 200mg/kg body weight daily for six weeks.

Group 4(HCC - Induced Treated)(Therapeutic): Animals were induced for pre-HCC (as group 2). After the induction of pre- HCC by DEN ,animals were post treated with AgNPs 200 mg/kg body for six weeks.

Group 5 (Preventive): Animals were pre-treated with AgNPs daily (200 mg/kg body weight) for six weeks before they were induced for pre- HCC(as group 2) .

All animals were sacrified at the end of experiment.

Histopathology

The liver tissues specimens were collected and fixed in 10% formalin and histological preparations were carried out then stained with H&E. processed by paraffin method, cut at six micrometers in thickness by using rotary microtome and stained with Hematoxylin and Eosin (H&E) (20). Sections were examind by histopathologist with olumpis Microscope (japan). Pholos were taken by digital camera (sony-japa 14 Migapixill).

Results :

Histopathological changes of liver are as follow. Control group, liver sections showed normal hepatic portal traid, central vein and normal hepatocytes (Figures 1).

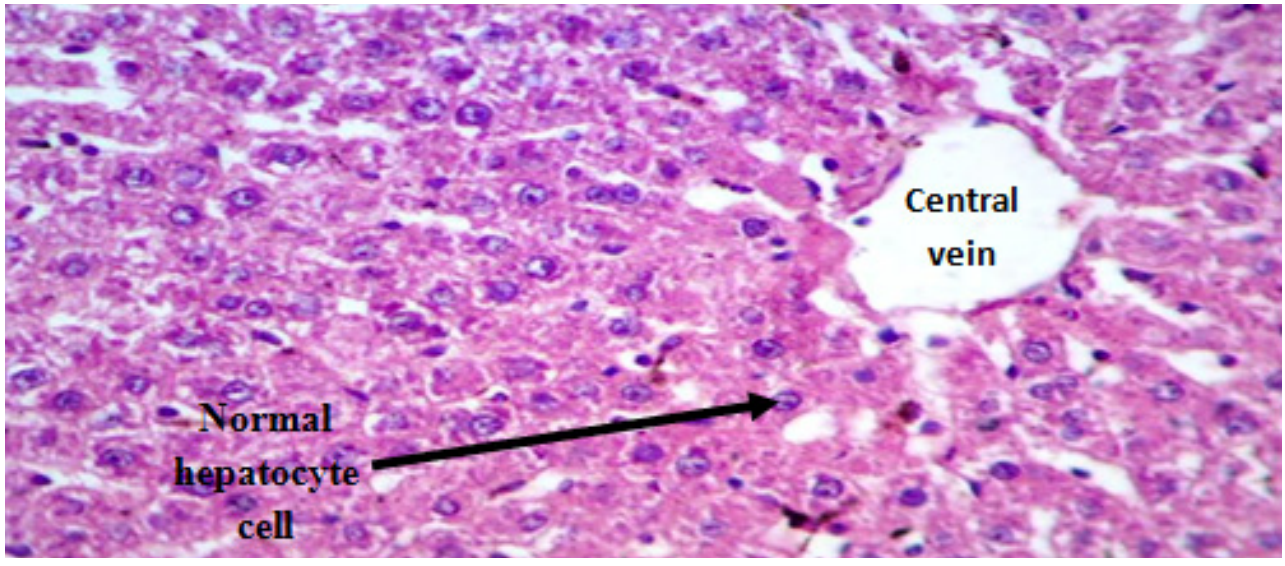

Fig -1- Rat liver section for control animals show normal hepatocyte, the hepatic tissue consist of central vein (C.V), hepatic cord.(40x H &E).

In group 2 ,DEN and CCl4 induced precarcinogenic (G2) groups, there were an extensive loss of hepatic architecture, severe necrosis, fatty change, certain hepatic cells showing atypical cells change, and bile duct proliferation (Fig 2 A and B).

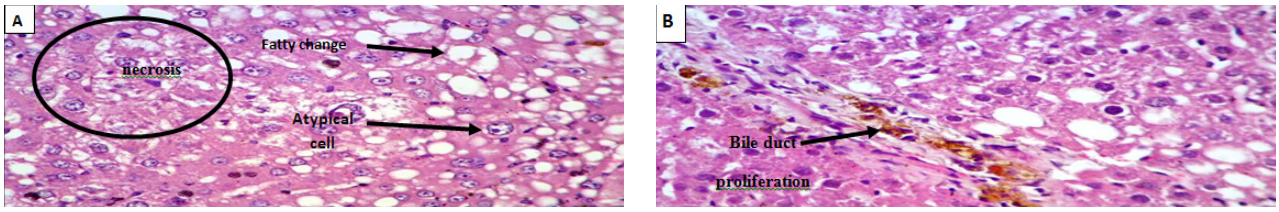

Fig 2 :Rat liver section, which DEN-Induced HCC(group2) showing necrosis, atypical cell and fatty change (A). bile duct proliferation(B). (40X H&E).

In group3,the section of liver tissue showed normal hepatocyte cell with mild necrosis (Fig3)

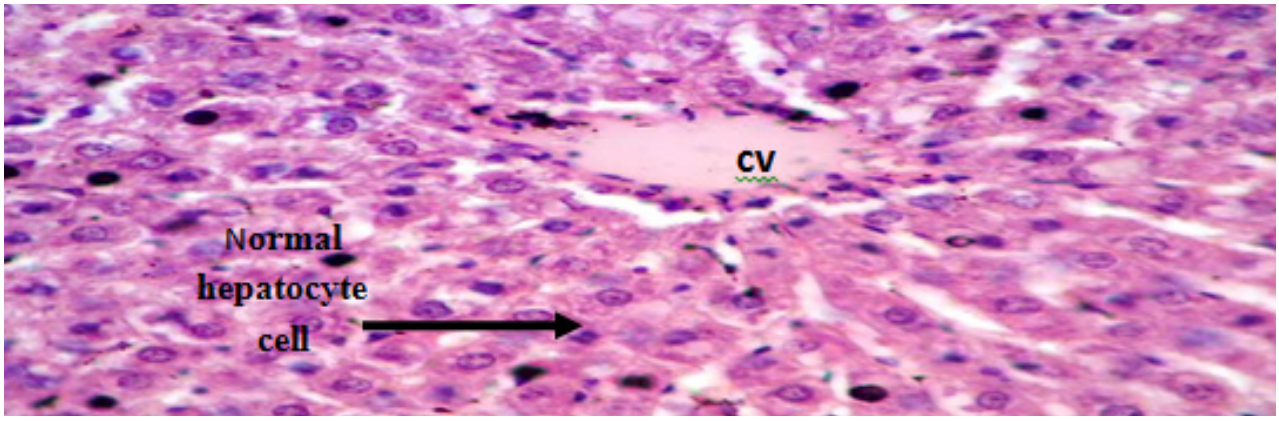

Fig 3:Rat liver section(group3) showing normal hepatocyte cell with mild necrosis (40X H&E).

In the therapeutic groups 4, the liver section shows certain area of necrosis and inflammatory cells infiltration ,congestion and mild fatty change, no atypical cells were seen(Fig 4).

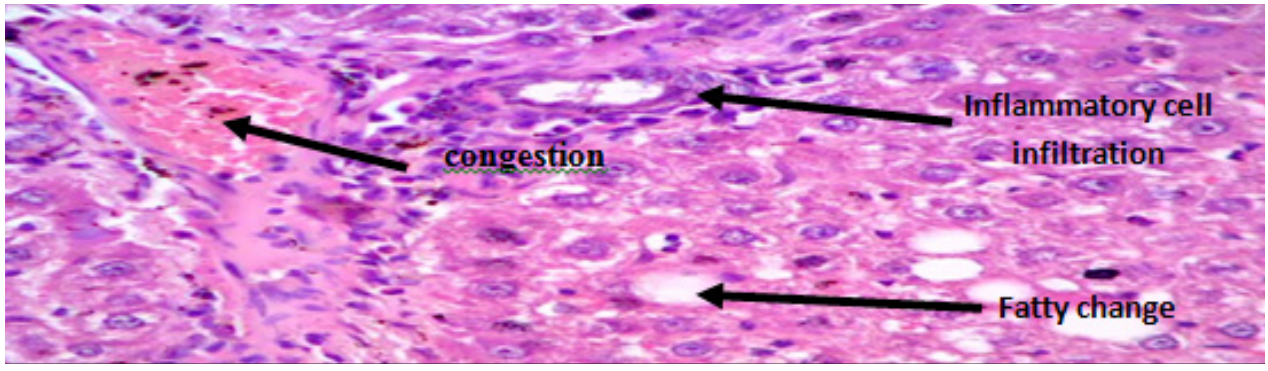

Fig 4:.Therapeutic Group4 : Rat liver section showing normal hepatocyte with mild necrosis, fatty change , congestion and Inflammantor cell Infiltration(40X H&E).

In group5 (preventive group), the liver section showing certain area of necrosis , inflammatory cells infiltration , mild fatty change and no atypical cells were seen (Fig 5).

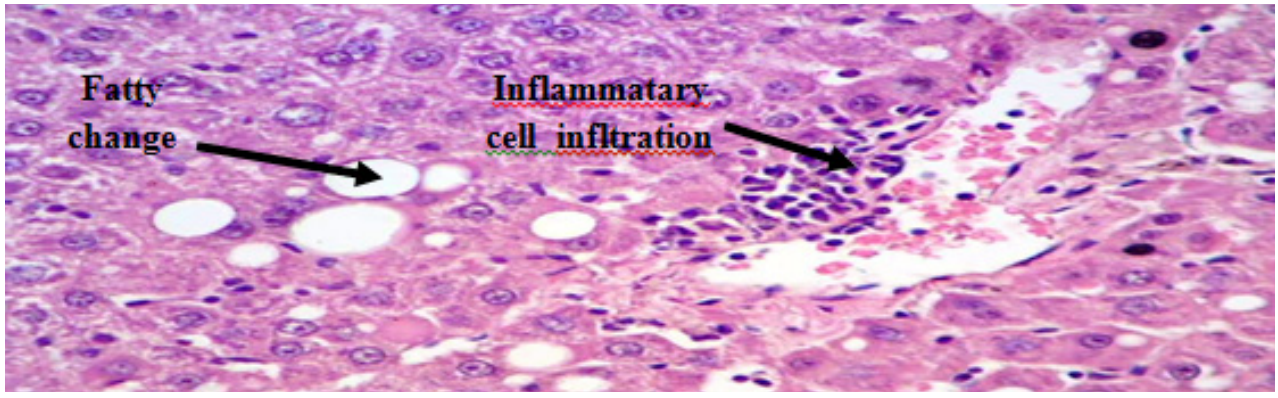

Fig 5: Group 5 Rat liver section showing normal hepatocyte with mild necrosis, fatty change and Inflammantor cell infiltration.

Disccusion:

The liver is the first organ to receive blood from the intestinal tract. A primary function of the liver is the biotransformation, detoxification, and excretion of xenobiotics, including carcinogens. The human liver is continually exposed to small doses of alkylnitrosamines, such as dimethyl-nitrosamine (DMN). These compounds are present in ordinary foodstuffs (probably to a far greater extent) result from nitrosation of amines in the gut(21). Hepatocellular Carcinoma can be induced in the livers of laboratory animals by a variety of chemicals such as diethylnitrosoamine (DEN) which is widely used chemical carcinogen in models of carcinogenesis of liver and esophagus . Hepatocellular carcinoma (HCC) is the third most common cause of cancer mortality worldwide (22).

Histopathological examination of liver detected that various alterations indicating the effect of silver nanoparticles including hepatocellular degeneration, necrosis and individual apoptosis were the most recognized hepatic changes that were dose dependent. Several studies confirmed that liver is the target organ for the effect of silver nanoparticles(23). Abdel-Hamid et al., studied the histopathological examination of liver biopsy. They were showed some of reversible cell injury as severe fatty change, inflammatory cellular infiltrate, atypical cells, and severe (cell death) necrosis. Such findings strongly suggest the ability of DEN to initiate hepatocarcinogenesis with the interactive effect of CCl4 (24). The histological features suggested that AgNPs is effective in reducing DEN-induced hepatocarcinogenesis in a dose dependant manner, (25).

Many attempts have been made to use AgNPs as an anticancer agent and they have all turned up to be positive (26). The size reduction of nanoparticles plays an important role in improving their bio-availability and compatibility for therapeutical applications in diseases like cancer (27). The developing more effective and less toxic anticancer agents, including natural products, is necessary to prevent or delay the process from hepatocarcinogenesis (28). Silver nanoparticles have been recorded to extend chemopreventive activities through controlling the tumor in vivo (29).

conclusion:

Silver nanopaeticles synthesized by the green method using Crocus sativus L can reduce the carcinogenic effect of diethylnitrosamine which induce hepatocellular carcinoma.

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دقائق الفضة النانوية المخلقة خضريا باستخدام مستخلص الزعفران تحد من تكون السرطانات في الكبد الجرذان

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الخلاصه:

جرى دراسة فدرة تاثير دفائق الفضة النانوية المصنعة بالطريقة الخضراء للحد من تسرطن الكبد في ذكور الجرذان البيضاء بعد استخدام المادة المسرطنة ثنائي مثيل نايتروز امين. ثلاثون من الجرذان البيضاء اوزانها تتراوح بين(200-150) غم وضعت في البيت الحيواني التابع للمركز العراقي لبحوث السرطان والوراثة الطبية. قسمت الى خمسة مجاميع كل مجموعة تتكون من ستة جرذان كانت المجموعة الاولى مجموعة السيطرة .المجموعة الثانية المستحثة لسرطان الكبد وذلك عن طريق الحقن البريتوني بجرعة واحدة من ثنائي مثيل نايتروز امين 200) ملغم / كغم من وزن الجسم)تليها حقن تحت الجلد)CCl43ملليتر/ كغم من وزن الجسم) لمدة 6 أسابيع. اما المجموعة الثالثة فقد تم الحقن البريتونى (200 ملغم / كغم من وزن الجسم)بدقائق الفضة النانوية لمدة 6 أسابيع. المجموعة الرابعة (المجموعة العلاجية) فقد تم حقن دقائق الفضة النانوية كما في المجموعة الثالثة بعد حقن المادة المسرطنة كما في المجموعة الثانية. واخير المجموعة الخامسة (الوقائية)فقد تم حقن الفضة النانوية كما في المجموعة الثالثة تتبعها الحقن بالمادة المسرطنة كما في المجموعة الثانية. ضحي بالحيوانات في نهاية التجربة واظهرت الدراسة النسيجية لخلايا الكبد في المجموعة الاولى ان نسيج الكبد طبيعي والمجموعة الثانية اظهر هناك تنخر وتغييرات دهنية و خلايا شاذة وانتشار القناة الصفراوية والمجموعة الثالثة اظهرت خلايا كبد طبيعية وتنخر متوسط .المجموعة الرابعة اظهرت تنخر و التهاب للخلايا و احتقان وتغييرات دهنية و المجموعة الخامسة اظهرت تنخر مع التهاب الخلايا وتغيير دهني.