Biosynthesis, Characterization and Bioactivity of Selenium Nanoparticles Synthesized by Propolis

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Summary

Biosynthesis of selenium nanoparticles is safe, inexpensive, eco-friendly, non-toxic materials, furthermore, is more stable due to natural coating of the organic molecules and do not aggregate with time, here by the present study designed to investigate the bioactivity and safety of a new biogenic Selenium- nanoparticles using synthesized by propolis. To evaluate biogenic Selenium-nanoparticles, 27 adult rats were divided in to 3 groups: first group consider as control(C), seconed group (T1) received inorganic selenium by orally administration in a dosage of 100µg /kg BW third group (T2) received Selenium nanoparticles orally in a dosage 100µg /kg BW for 4 weeks. Body weight, food intake, blood Selenium binding protein, Catalase, and liver enzymes Aspartate Amino Transferase and Alanin Amino transferase were evaluated two weeks interval. Results showed that body weight and food intake were elevated significantly during the experiment periods. Rats had selenium nanoparticles showed significant increase in Selenium binding protein and Catalase correlated with normal values of liver enzymes. It can be concluded that the new propolis mediated Selenium- nanoparticles improve the availability of the Selenium to its binding proteins that limit its toxicity when compare with inorganic selenite.

Keywords: Selenium, Nanoparticles, Biosynthesis, Propolis.

Introduction

Selenium is an essential trace element (1) that evinces antioxidant activity (2), anti-inflammatory (3), antimutagenic (4), anticarcinogenic (5 and 6) or chemopreventive (7) ,antiviral (8), antibacterial (9), antifungal (10 and 11). Furthermore, it is an integral component of selenoproteins that participating in a whole series of physiologically important processes (12). In spite of its physiological importance, presence of free selenium has toxic effects to cells. Therefore, productions of Se in nano molecules gate the priority for researchers in this field. Selenium in nano form has shown excellent biological activities with no toxic effects (13). There are three different approaches that can be used for synthesis of selenium nanoparticles (SeNPs) covering the physical (14), chemical (15), and biosynthesis techniques (16). Large number of plant types were reported for nanoparticles synthesis (17 and 18), Leaf extract of Capsicum annuum reduces SeO_3^{-2} to red color indicating formation of selenium nanoparticles (SeNPs) (19). Dried fruit extract of Vitis vinifera can synthesize spherical SeNPs in the range of 3–18 nm (20). Polysaccharides extracted from Undaria pinnatifida, edible seaweed, enhance the stability of SeNPs (21 and 22). Biosynthesis of SeNPs achieved by reducing bacteria, Gram negative bacteria such as Pseudomonas aeruginosa (23), Klebsiella pneumonia (24) and by gram positive bacteria, such as Bacillus subtilis (25), Lactobacillus acidophilus (26). There are reports on SeNPs synthesis by fungi like Actinomycetes (27 and 28). The biogenic SeNPs are stable due to natural coating of the organic molecules and do not aggregate with time, whereas external addition of stabilizing agents is required in chemical synthesis (29). Propolis is a resinous hive plant sources product that's collected by honey bees, it's contain many compounds like resin, and waxes, essential oils, amino acids, ethanol, minerals, vitamins A, B complex, E, Flavonoids and phenolic compounds(30). In addition propolis used safely for its anti-oxidant, anti-inflammatory (31), cardio-

protective, hepato-protective, and Neuro-protective properties (32).

Producing nanoparticles using propolis depends on the reduction power it possesses due to the presence of many active groups which have reducing activity, this is one of the modern methods. Propolis proved to be efficient in producing iron oxide nanoparticles that showed strength in the treatment of iron deficiency anemia (33). The present experiment was designed to evaluate the bioavailability of the Se in the experimental animals delivered from Se nanoparticles synthesized by propolis The present study designated to evaluate the bioactivity and safety of the new biosynthesized Se-nanoparticles in compare with inorganic selenite.

Materials and Methods

Biosynthesis of Selenium nanoparticles: In a conical flask, containing 300 ml of propolis watery extract solution, 150 ml of sodium selenite solution added slowly drop by drop. The flask was covered by a layer of aluminum foil to avoid the effect of light on the nanoparticle composition process. The pH of the mixture was adjusted to 8 by the addition of drops of 1 N of NaOH, and stirred for 6 hours at 37 - 40 Cº. The prepared nanoparticles were separated by centrifugation at 12000 rpm for 30 minutes in 20 Cº **.**

UV-Visible Spectra Analysis: The spectra of the synthesized Selenium nanoparticles were analyzed by UV – visible spectroscopy Shimadzu UV – 1600, Japan, with wave rang 190.00 – 1100.00 nm, scan speed of sampling interval was 0.5min (22).

Fourier Transform Infrared (FTIR) Spectroscopy: The prepared SeNP analyzed by FTIR spectroscopy (ABB/spectro-lab/MB3000/UK), Laser phase and the F , D amplitude 35 and the rejected scan counter about 24, FTIR characterization was carried out under classic KBr pellet technique that measures infrared intensity vs wavelength (wave number) of light from 400 – 4000 cm, it is used to determine the nature of associated functional groups and structural features of biological extracts with nanoparticles **.**

The prepared particles composition were examined using X-ray diffraction (XRD) (6000/Shemadzu Japan, XRD patterns were calculated using X'per Rota flex diffraction meter using Cu K radiation and λ =1.5406 A°, 40.0 kV voltage, 30.0 ma x-ray current, the measurement of XRD obtain in theta – 2 theta, continues scan, rang (20.000 – 60.000 deg) speed about 50000 deg/min in 0.60 sec. Crystallite size is calculated using Scherrer equation (CS) as described by (34).

Equation 1 $CS= K\lambda /\beta \cos \theta$

Where CS is the crystallite size Constant [K] = 0.94 β is the Full Width at Half Maximum (FWHM).

The SeNP were examined by Scaning Electron Microscopy (SEM) (Tescan Vega lll /Czech) to study the morphology and shape of the particles, to get the best view under SEM, samples were slightly pressed into pellets at 0.5 ton-load. SEM MAG 30.0 Kx, SEM HV 10.0 kV and the image were exanimated at 2 - 10µm scale(35). Bioactivity and safety of SeNP.

Twenty seven healthy male Wistar albino rats (Rattus norvegicus albinus) 3.5 months old with an average weight between 200 -230 gm were used. The animals were kept in appropriate plastic cages with 12 hours day/night and kept in Controlled room temperature at 22°C±3°C and humidity (50±10%), and were permitted ad libitum access to water and rodent's laboratory pellets.

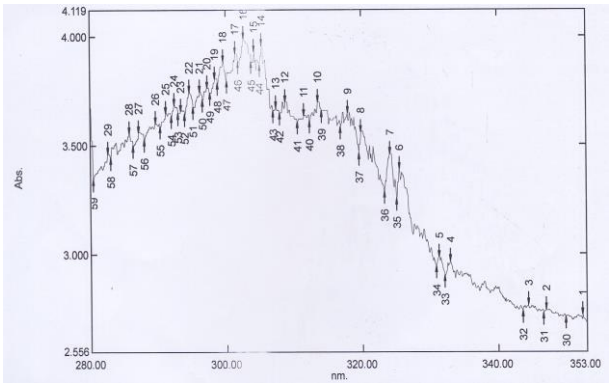
The experimental rats were divided into 3 groups each of three replicants with 3 animals. First group control, given orally D.W. second group given orally (gavage) sodium selenite (0.1 mg /kg body weight) (36). The third given orally (gavage) selenium nano particles(0.1mg Se/kg BW) . The experiment lasted for 30 consecutive days, data were recorded weekly interms of body weight and food intake along of experiment. Blood samples were collected every two weeks for estimation of Catalase , Selenium binding protein 1 using ELISA Kit manufactured by Wuhan Fine Biotech Co., Ltd., China., Liver enzymes AST and ALT.

Results and discussion

During the addition of selenium solution to the propolis solution, appearance of brick –red color indicate the formation of SeNPs as shown in (Fig.1) (tube C. Red color) is the characteristic indication of Se nanoparticles (37). This color formed as a result to the excitation of the surface Plasmon vibrations of the monoclinic Selenium particles providing an improvement for the formation of Se oxide nanoparticles. The reduction of selenium from its salts to produce selenium oxide particles depends on the reducing molecules that are formed the propolis.

Figure, 1: Color changes during biosynthesis of Se- Nanoparticles using propolis extract after mixing of Selenite solution to propolis extract in: 1:2 ratios. A: Selenite solution.

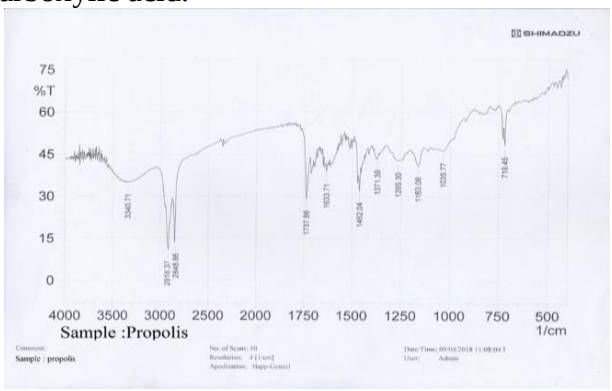
Analysis of UV –Visible showed the different peaks of absorption values between 280-353 nm (UV range) in (Fig.2). The most well defined peak at 300 nm represent the surface plasmon resonance of the nanoparticles formed from Selenium using propolis. The present results concurred with the findings of (38) others reported that the absorption band between 200-300 nm for the nanoselenium synthesized using Klebsiella pneumonia. Similar observations were reported by (39 and 40) who synthesized nanoselenium using Pseudomonas alcaliphila and Saccharomyces cerevisiae respectively which exhibited absorption band between 200-300 nm A band observed in the spectrum, corresponding to surface plasmon resonance indicating the formation of Se NPs (20 and 41) the smallest molecules give the highest peak at UV spectroscopy between 280-320 nm.

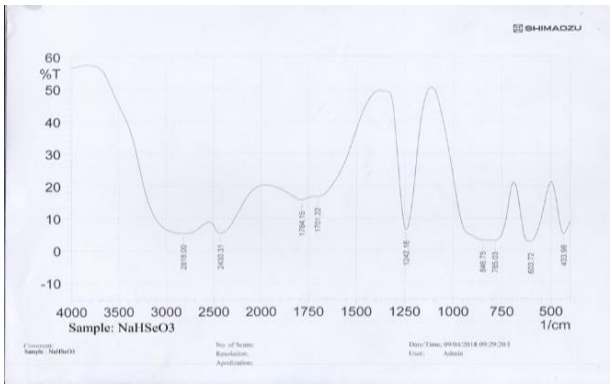


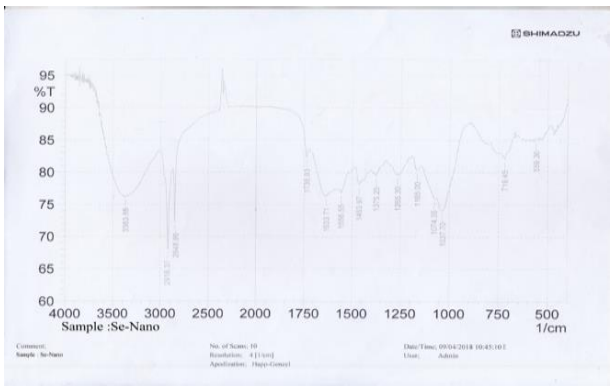
Figure, 2: The ultraviolet- visible spectroscopy analysis of the Se- nanoparticles prepared using propolis .

The FTIR for the propolis extract, sodium selenite solution and the SeNPs formed from the mixture were shown in (Fig.3). The synthesized SeNP were characterized by FTIR in order to investigate the biological compounds responsible for the synthesis and stability of the particles. The analysis of the chromatographic images of the FTIR for the present prepared compound revealed appearance of many peaks indicating different vibrations due to the different IR absorption.The wavelength (cm) were divided into different regions. Each region of wave length indicates the presence of bounded molecules. Results revealed that the prepared Selenium nanoparticles using propolis extract containing compounds rich with O-H, C-C, N-H and COOH at IR absorption 3400- 1500cm-1.The result shows sharp absorption peaks at3363.86 cm-1 can be assigned to –OH in alcohols and phenol (42). The peak of 2916.37 cm-1 corresponds to aliphatic saturated C-H stretching modes, the peak at 2848.86 cm-1 corresponds to CH3 attached to O or N (OCH3) group (43). The peak at 1463.97 cm-1 corresponds to CH2 CH3 in aliphatic compound and /or carboxylate group,(antisymmetric stretching vibration) the peak at 1375.25 cm-1 corresponds toCH3 in aliphatic compound, the peak at1265.30 cm-1 corresponds to C-O-S, C-N in aromatic amine , the peak a1165.00 cm-1 corresponds to SO2 and NO2 in sulphones. Another broad feature was observed between 1074 and 1038 cm−1 , which corresponds to C-O-C and C-H stretching arising from the carbohydrate groups (44 and 45), the peak at 719.45 cm-1 corresponds to CH2 in hydrocarbons and the

peak at 559.36 cm-1 corresponds to C-C=O in carboxylic acid.

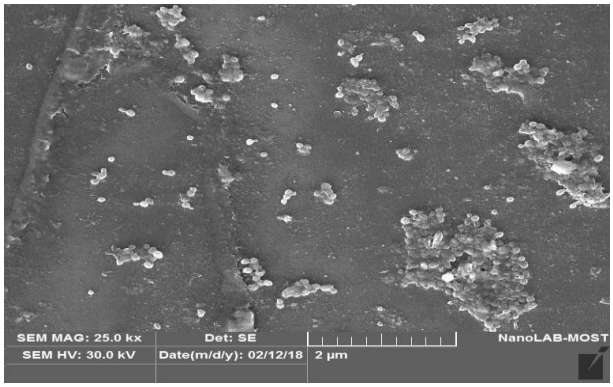


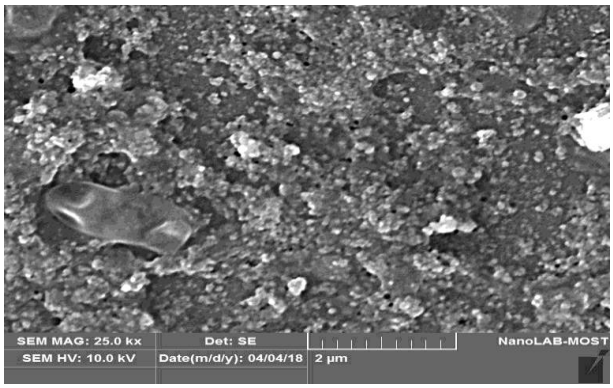




Figure, 3: FTIR spectrum illustrates the functional groups of propolis, NaHSeO3 and Se -Nano particles.

The result of the analysis of the SEM micrographs represented in (Fig.4). Images showed accumulation of spherical, amorphous nanoparticles with different sizes between 30 - 36 nm. Selenium nanoparticles prepared by propolis.





Figure, 4; Morphological and size characterization of Selenium Nanoparticles.

The result of the analysis of the SEM micrographic image represented in(Fig. 4) was employed to analysis the morphology and size of selenium nanoparticles that were formed in the presented study. This outcome can be explained by the fact that the polyphenol concentration in propolis extract plays roles in the formation of the final structure and the particles size of these biosynthesized nanoparticles (46).

The X-ray diffraction pattern of nanoselenium is shown in (Fig.5), the diffraction peaks at 2θ (degrees) of 23.51°, 29.50°, 41.30°, ,45.35° and 48.10° were indexed as the (55,101,110,111 and 200) planes of Se respectively. All the diffraction peaks in the 2θ range measured corresponded to the hexagonal structure of Se with lattice constants a = 4.3662Å and c = 4.9536Å and were in good agreement with those on the standard data card. The sharpness of the diffraction peaks revealed that the product had well crystallized nature of selenium nanoballs with broad diffraction peaks at low angle hence confirming the amorphous/hexagonal nano crystalline nature of the sample (47).

The crystallite size of selenium was calculated using Scherrer's equation**.**

$$D= K\lambda / \beta \cos \theta.$$

Where D is the grain size, K is the constant taken to be 0.94, λ is the wavelength of the X-ray radiation, β is the line broadening at half the maximum intensity, θ (Bragg angle) is the angle of diffraction. Similar XRD patterns had been found by (20) when using dried Vitis vinifera (raisin) to prepare SeNPs.

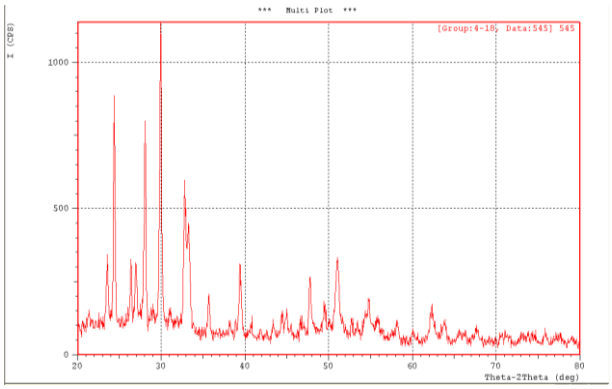


Figure 5: XRD spectrum of nanoparticles synthesized by propolis extraction.

The Body weight gain in selenium nanoparticles treated group (T2) significantly increased (p≤0.05) during 2 weeks of the experiment than the control group and selenite treated group (T1) as showed in (Table, 1), also the body weight gain in selenium nanoparticles treated group (T2) significantly increased (p≤0.05) during 4 weeks of the experiment than the control group and selenite treated group (T1) as showed in (Table,1) this table also showed significant differences (p≤0.05) between the two times of the experiment represented by capital litters in selenium nanoparticles treated group (T2).The food intake in selenium nanoparticles treated group (T2) significantly increased (p≤0.05) during 2 weeks of the experiment than the control group and selenite treated group (T1) as showed in (Table,1), also the food intake in selenium nanoparticles treated group (T2) significantly increased (p≤0.05) 4 weeks of the experiment than the control group and selenite treated group (T1) as showed in (Table,1) this table also showed significant differences (p≤0.05) between the two times of the experiment represented by capital litters in all the three groups**.**

Body weight and food intake evaluation reflect the health situation of the individual, Selenium is one of the trace metals that play an important role in the proper functioning of carbohydrate and lipid metabolism. Some of the trace metals are thus essential for maintaining homeostasis, while deficiency of these trace metals can cause disorders with metabolic and physiological imbalances. Selenium found to produce effects similar to those produced by insulin (48). The increased body weight changes that found in rats received selenium nanoparticles reflect the increase in body mass.The exactly mechanism that Selenium could induce increase in body mass could be attributed to the alteration in hepatic fatty acids and energy metabolism via increase the transcription of the Glut2 in adition to the enzymes involved in lipid metabolism (49). Although Selenium supplementation dove required levels of the individual may reduce metabolism of body and reduced food intake and body weight gain (50) but the prepared SeNPs showed different effects improving metabolism and increased body weight mass and food intake.

Recent studies indicated that organic selenium preparation like Se-yeast produced improvement of food intake and body weight gain (51). The present results in regard to increased body weight and food intake indicted that prepared Se- nanoparticles could enhance digestibility of nutrients as found by (52)) when they fed broilers to nano-se there were improvement in growth and nutrient digestibility. Increased body mass could be resulted from increased in protein synthesis caused by the Se-nanoparticles papered by propolis, since it was documented that available selenium may increase proteins synthesis by increasing activation of different cellular metabolic pathways resulting in increased mRNA(53).

The serum aspartateamino transferase AST and alanin amino transferase ALT enzyme activity in selenite treated group (T1) significantly increased (p≤0.05) during 2 and 4

weeks of the experiment than the selenium nanoparticles treated group (T2) and control group as showed in (Table, 2) also showed no significant differences ($p \leq 0.05$) in the activity of two enzymes along experimental period.

Serum Catalase activity in selenite treated group (T1) significantly increased ($p \leq 0.05$) during 2 weeks of the experiment than the selenium nanoparticles treated group (T2) and control group as showed in (Table, 3) also Catalase enzyme in selenite treated group (T1) significantly increased ($p \leq 0.05$) during 4 weeks of the experiment than the selenium nanoparticles treated group (T2) and control group as showed in (Table, 3) **.**

Selenium binding protein1(SBP1) in selenium nanoparticles treated group (T2) significantly increased ($p \leq 0.05$) during 2 and 4 weeks of the experiment than selenite treated group (T1) and control group as showed in (Table, 3).

The AST , ALT were measured to assess the possible interaction of Selenium nanoparticles with liver. The significant elevation in serum AST and ALT in rats supplemented with inorganic selenite indicated hepatocytes structural damage. In case of liver cells damage these enzymes leak to the circulation (54 and 55). Some animal studies have indicated that liver is the main target organ of selenium toxicity (56). Damage to the structural integrity of the liver is assessed by elevated serum levels of enzymes such as AST, ALT. Liver and kidney damage and impaired immune .

Responses have been reported in rodents following oral exposures to Se compounds (57). Selenium is present in all tissues, with the highest accumulation found in the liver, spleen, and pancreas(58) Elevated concentrations of hepatic Se were associated with increased levels of plasma AST and ALT (59). On the contrary, Se-nanoparticles prepared by propolis showed less effects on hepatocytes and circulated AST and ALT activity in a similer way of organic selenium (60). Our results denoted the less toxic effects of selenium nanoparticles as oxidative stress inducer for hepatocytes (61). In addition it may cause less apoptosis of liver cells than did the selenite (62). Murine toxicology data from other studies indicate that selenomethionine (SeMet) and SeNPs possess a lower cytotoxicity than selenite (63 and 64). Catalase enzyme reflect the protecting activity of the cell against oxidative stress caused by reactive oxygen species, it catalyses hydrogen peroxide into water and oxygen (65). Catalase usually locate within the peroxisome organelle (66). The present Catalase increase in selenite group could resulted in response to an oxidative stress induced by selenium especially of liver cells, since catalase is a key enzyme for the resistance against an oxidative stress (67 and 68).

Also selenium binding protein 1 results showed significant differences in selenium nanoparticles treated group compared with selenite treated group and control respectively, which convenient with several studies because of availability of selenium nanoparticles, this convenient with studies suggest that Nano-Se can serve as an antioxidant with reduced risk of selenium toxicity (69). In many studies that applied SeNPs, increasing selenoproteins and selenoenzymes activity was reported with lower toxicity in comparison to an organic or inorganic form of Se (70). Also experimment carried out by (71) confirm that the selenium nanoparticles were able to restore cell structure and/or prevent cellular damage. Short-term, high-dose selenite caused more pronounced oxidative stress, greater liver injury, and pronounced retardation of growth compared with SeNPs (14). Finally this study confirmed that the organic propolis biomediated SeNPs has fewer negative effects than the inorganic form. This leads us to the possibilities of various modifications of the surface of particles is an additional advantage of using these particles, rather than traditional inorganic forms, and propolis biomediated SeNPs can be employed with better physiological performances. This study concludes that the propolis biomediated SeNPs synthesis exhibit high bioactivity and safety more than the traditional inorganic form.

Table, 1: Body weight gain (g/ animal / two weeks) and Food intake (g/ animal / two weeks) under effect of biogenic Se- Nanoparticles in compare with selenite solution during 4 weeks, Means ±SE. n- 6.

Experimental groups	Body weight gain		Food intake	
	2weeks	4weeks	2 weeks	4 weeks
Control	28.00 ± 1.39 A b	30.16 ± 0.70Ab	159.48 ± 3.86B b	179.80 ± 4.74Ab
T1	20.10 ± 0.33A c	20.66 ± 0.33A c	116.33 ± 2.09B c	126.62 ± 2.05Ac
T2	36.83 ± 0.47Ba	41.83 ± 0.70A a	211.44 ± 2.58B a	238.36 ± 0.97Aa

T1: Selenite treated group, T2 Selenium Nanoparticles treated group. Capital letters denote significant differences (P≤0.05) within group (rows).Small letter denote significant differences (P≤0.05) between groups (column).

Table, 2: Serum aspartate amino transferase (AST) activity (U/L) and Serum alanin amino transferase (ALT) activity (U/L) under effect of biogenic Se- Nanoparticles in compare with selenite solution during 2 and 4 weeks , Means ±SE. n- 6.

Experimental groups	AST (U/L)		ALT (U/L)	
	weeks 2	4weeks	2 weeks	weeks4
Control	136.55± 1.54 Ab	134.24± 1.23A c	42.98 ± 1.45A c	43.82 ± 1.45A c
T1	142.50 ± 1.42A a	144.46 ± 0.64A a	59.69 ± 0.63A a	60.77 ± 0.70A a
T2	136.56 ± 1.14A b	138.21 ± 0.92A b	46.90 ± 1.11A b	47.74 ± 1.11A b

T1: Selenite treated group, T2 Selenium Nanoparticles treated group Capital letters denote significant differences (P≤0.05) within group (rows) .Small letter denote significant differences (P≤0.05) between groups (column).

Table, 3 serum catalase activity (U/ml) and Serum Selenium binding protein (U/ml) activity under effect of biogenic Se- Nanoparticles in compare with selenite solution during 2 , 4 weeks, Means ±SE. n- 6.

Experimental groups	Catalase (U/ml)		Selenium binding protein1 (U/ml)	
	weeks2	4weeks	2 weeks	weeks4
Control	394.86 ± 12.64A b	409.53 ± 13.37A b	492.74±11.69Ab	508.49±11.74Ab
T1	502.42 ± 6.45A a	523.54 ± 6.80A a	549.80±8.77Ba	A611.77±8.95Aa
T2	404.91 ±5.82A b	412.20 ± 7.10A b	561.02±12.04Ba	A631.68±10.59Aa

T1: Selenite treated group, T2 Selenium Nanoparticles treated group Capital letters denote significant differences (P≤0.05) within group (rows) .Small letter denote significant differences (P≤0.05) between groups (column).

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التصنيع البايولوجي والصفات والنشاط الحيوي للسلينيوم النانوي باستعمال العكبر

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الخلاصة

يعتبر التصنيع البايولوجي للسلينيوم النانوي أمن ورخيص وصديق للبيئه ومن المواد غير السامة وعلاوة على ذلك يكون السلينيوم النانوي مستقرا بسبب احتوا ئه على الغطاء الطبيعي من المواد العضويه والتي تمنع من حدوث تراكمه مع مرور الوقت ، صممت هذه الدراسه للتحقق من التوافر البايولوجي لنانوات السلينيوم المحضرة بطريقه جديده باستعمال العكبر . تم استعمال 27 جرذا بالغا وقسمت الى ثلاث مجاميع متساويه ، المجموعه الاولى اعطيت الماء المقطر عن طريق التجريع الفموي (مجموعه السيطرة) والمجموعه الثانية اعطيت السلينوم اللاعضوي عن طريق التجريع الفموي وبجرعه مقدارها 100مايكروغرام لكل كيلو غرام من وزن الجسم ، بينما اعطيت حيوانات المجموعه الثالثه سلينيوم نانوي عن طريق التجريع الفموي بجرعه مقدارها 100مايكروغرام لكل كيلو غرام من وزن الجسم استمرت التجربه لمده اربع اسابيع تم خلالها حساب الزياده الوزنية للجسم والعلف المستهلك وحساب تركيز البروتين المرتبط بالسلينيوم 1 ،تركيز انزيم الكتاليز وتراكيز انزيمات الكبد في مصل الدم كل اسبوعين . أستنتج من ذلك ان استعمال السلينيوم النانوي المصنع باستعمال العكبر ادى الى تحسين التوافر البيولوجي للسلينيوم بواسطه ارتباطه بالبروتين الذي ادى الى قله اثاره السميه عند مقارنته بالسلينيوم اللاعضوي .

الكلمات المفتاحية:السيلنيوم، النانوي،التصنيع البايولوجي،العكبر.