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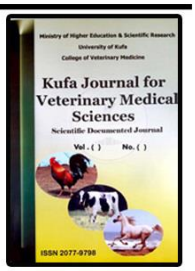

The effect of black currant selenium nanoparticles on dyslipidemia and oxidant-antioxidant status in D- galactose treated rats

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Abstract:

The current study was aimed to explore the effect of black currant selenium nanoparticles (BCSeNPs)on serum lipid profile and oxidant- antioxidant state markers in blood of D- galactose(D-gal) treated rats. The green synthesis of SeNPs as described earlier in our previous study was characterized by color changes; Ultraviolet- visible (UV–VIS) spectroscopy; scanning electron microscopy (SEM) techniques; X–ray diffraction analysis (XRD); Fourier transform infrared spectroscopy (FTIR). The results revealed prevalence of brick- red color of BCSeNPs characterized by spherical crystals with average particle size in the range of 18-50 nm. Thirty-two (32) adult male rats were divided randomly and equally into four experimental groups (8/group) and handles as follows for eight weeks: Control (C) group: rats in this group were treated with intra peritoneal injection (*i.p*) and oral intubation of normal saline. T1 group: animals in this group were subjected to *i.p*. of D gal a dose (150 mg/kg/day), which was dissolved in normal saline solution. T2 group: the rats were oral intubation BCSeNPs (1mg/ Kg. B. W). T3 group: rats in this group were administered BCSeNPs concurrently with *i.p*. of D-gal in the same previous doses. Blood samples were collected from heart by cardio puncture technique at 2nd and 8th weeks of the experiment and serum samples were used for estimation of some biochemical parameters related to oxidant-antioxidant status markers: Malondialdehyde (MDA) and Total antioxidant capacity (TAO-C); Serum lipid profile: concentration of total cholesterol(TC), triglyceride(TAG), high density lipoprotein-cholesterol(HDL-c), low density lipoprotein- cholesterol (LDL-c), and very low density lipoprotein-cholesterol(VLDL-c). At the end of experiment, after animal scarifying, section from liver was taken for detection of gene expression of glutathione peroxidase.

The results of here in study confirmed a case the oxidative stress and hyperlipidemia in T1 group manifested by significant depression in serum TAO-C concentration and decrease in glutathione peroxidase gene expression level. While, caused elevation in serum MDA concentration and significant decrease in serum concentration of HDL-c with significant elevation in serum concentration of TAG, VLDL-C, TC, and LDL-c. At the end of experiment, BCSeNPs intubation in T2 group caused alleviation of previous mentioned parameters related to oxidative stress and lipid profile. **On conclusion,** the result in a current study showed that black currant selenium nanoparticle has both a preventive and a therapeutic role in D-gal toxicity in adult male rats, where BCSeNPs can be considered as antioxidant and hypolipidemic agent.

Key Words: Selenium nanoparticles, D-galactose, lipid profile, glutathione peroxidase.

تأثير جسيمات السيلينيوم النانوية- الزبيب الأسود على حالة اضطراب الدهون و حالة الاكسدة - مضادة الاكسدة في لجرذان المعالجة ب د- كالاكتوز

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الخلاصة:
هدفت الدراسة الحالية لمعرفة تأثيرجسيمات السيلينيوم النانوية المصنعة باستخدام الزبيب الأسود (BCSeNPs) على الصورة الدموية للدهون وبعض المعايير الخاصة بالأكسدة ومضادة الأكسدة في دم جرذان المعالجة بـ د-كالاكتوز (D-gal).تم تميز التصنيع الحيوي لجسيمات السيلينيوم النانوية المرتبطة مع الزبيب الاسود بواسطة تغيرات اللون؛ استخدام التحليل الطيفي المرئي للأشعة فوق البنفسجية (UV-VIS) ؛ تقنيات المسح المجهري الإلكتروني (SEM) ؛ تحليل حيود الأشعة السينية (XRD) ؛ التحليل الطيفي بالأشعة تحت الحمراء فوريه (FTIR). أوضحت النتائج ظهور لون أحمر قرميد من BCSeNPs يتميز بالبلورات الكروية بمتوسط حجم الجسيمات في حدود 18-50 نانومتر.تم تقسيم اثنان وثلاثون (32) من ذكور الجرذان ويستير البيضاء البالغة بوزن (200 ± 10 غم) بشكل عشوائي وبالتساوي الى أربع مجموعات(ثمانية/مجموعة) وكان تعامل يومياً ولمدة ثمانية اسابيع كالتالي: مجموعة السيطرة :(C)عولجت الجرذان في هذه المجموعة عن طريق الحقن داخل الصفاق والتجريع الفموي بمحلول ملح طبيعي. مجموعةT1: تعرضت الجرذان في هذه المجموعة للحقن داخل الصفاق بجرعة (150ملغ/كغم من وزن الجسم) والذي تم اذابته في محلول ملحي طبيعي . في حين تم تجريع الجرذان في مجموعة T2 جسيمات السيلينيوم النانوية فمويا وبجرعة (1ملغ/ كغم من وزن الجسم). اما حيوانات المجمزعة T3 فقد جرعت فمويا جسيمات السيلينيوم النانوية و حقن د-كلاكتوزداخل الصفاق بنفس الجرعة المذكورة سابقاً .تم جمع عينات الدم عن طريق ثقنية ثقب القلب في الأسبوعين الثاني والثامن من التجربة ، وتم تحضير عينات المصل لقياس الاختبارات البيوكيميائية المتعلقة بمؤشرات حالة الأكسدة والمضادة للأكسدة: المالونداياالدهايد (MDA) والسعة الكلية لمضادة الأكسدة (TAO-C) ؛ الصورة الدموية للدهون:تركيز الكوليسترول الكلي (TC) ، الدهون الثلاثية (TAG) ، الكولسترول في البروتين الدهني عالي الكثافة (HDL-c) ، الكولسترول في البروتين الدهني منخفض الكثافة (LDL-c) ، الكولسترول في البروتين الدهني الواطئ الكثافة جدا (VLDL-c). تم التضحية بالحيوانات في نهاية التجربة ، تم أخذ جزء من الكبد للكشف عن التعبير الجيني للكلوتاثيون بيروكسيديز. أكدت النتائج هنا في الدراسة حالة الإجهاد التأكسدي وفرط شحميات الدم في مجموعة T1 يتجلى في انخفاض كبير في تركيز TAO-C في المصل وانخفاض مستوى التعبير الجيني للكلوتاثيون بيروكسيديز. بينما تسبب ارتفاع في تركيز MDA في المصل وانخفاض كبير في تركيز مصل HDL-c مع ارتفاع كبير في تركيز المصل لـ TAG و VLDL-C و TC و LDL-c. في نهاية التجربة ، في حين تسبب تجريع BCSeNPs في مجموعة T2 في تقليل حدة المعايير السابقة الذكر المتعلقة بالإجهاد التأكسدي وصورة الدهون. نستنتج من الدراسة الحالية الدور الوقائي والعلاجي لجسيمات السيلينيوم النانوية-الزبيب الأسود ضد سمية D-gal في ذكور الجرذان البالغة, حيث يمكن اعتبار BCSeNPs كعامل مضاد للأكسدة وخافض للدهون .

الكلمات المفتاحية: جسيمات السيلينيوم النانوية ، د-الكالاكتوز ، الصورة الدهنية ، الكلوتاثيون بيروكسيديز

Introduction

Nanotechnology sciences provide the improvement of experimental practice for the preparation of the nanoscale constituents with exceptional possessions **(1).** Today, production of nanoparticles (NPs) using biosynthetic techniques, has been considered as a valuable method with increasing attraction (**2,3**). Biogenic synthesis of Se nanoparticles is frequently achieved by reduction of selenate/selenite in presence of bacterial proteins or plant extracts containing phenols, flavonoids amines, alcohols, proteins and aldehydes **(4,5).** Nano-Se possesses better antioxidant capability than other chemical forms of selenium while reducing the risk of selenium toxicity. They appear to have physiological properties, of important antioxidant effect (**6,7**). Compound SeNPs protected the liver and kidney against acetaminophen toxicity through reducing oxidative stress, enhancing endogenous anti-oxidants and protecting mitochondrial functions **(8)**. Its role in treatment of liver disease and as antidiabetic is well documented (**9,10).** Treatment by SeNPs, is essential to improve health and performance, oral SeNPs supplements showed no disadvantages and its well tolerated by all patients **(11).** The role of selenium nanoparticles in mitigation of high temperature- stress is enhanced by their antioxidant defense system **(12).** D-gal is a reducing sugar and can be metabolized at

normal concentration. However, at high levels, it induces the production of reactive oxygen species (ROS) and advanced glycation end products **(**AGEs) **(13,14)**. It has been suggested that AGEs binding to its receptor form advanced glycation end products (RAGE) in many cell types induces pathophysiological cascades linked to the downstream activation of NF- κB and other signaling pathways that lead to ROS generation and certain proinflammatory responses **(15,16,17).** Besides, ROS generation by D-gal could induced memory, systemic disfunction and neuroinflammation **(18).** The role of D-gal in inducing aging associated change such as increased oxidative stress, decreased antioxidant enzyme activity and mitochondrial function (**19-23**) has been well illustrated.

2- Materials and Methods:

Green synthesis of BCSeNPs: using black currant aqueous extract was prepared as described by **(24,25).** Characterization of BCSeNPs were performed by: Ultraviolet–visible spectroscopy (Metertech SP-8001 Taiwan) as described by (**26,27);** X-ray diffraction (Shemadzu-6000 Japan)as describe by **(28,29**); Scanning Electron Microscope(SEM-Tescan Vega III, Czech) as described by **(30)**; Fourier-transform infrared spectroscopy (Shimadzu8400s, Japan) as described by (**31,32**). The current study had executed in the animal house vassal to the college of Veterinary Medicine, AL-Qadisiya University through the period expanded from January, 2019 to march, 2019. Mature male Wistar rats (aged: 90 days, weighted: 190 ± 5.5 g) have been utilized in the current study. After acclimatization, thirty two (32) adult male rats were divided randomly and equally into four experimental groups and handles as follows for eight weeks: control(C) group : rats in this group were treated with intra peritoneal injection and oral intubated of normal saline, D-galactose(D-gal) (T1) group: animals in this group were subjected to intra peritoneal injection of D-gal a dose (150 mg/kg/day), black currant- selenium nanoparticles(BCSeNPs)(T2)group: the rats were intubation black currant- selenium nanoparticles (1mg/Kg.B.W) and (T3)group: rats in this group were administered BCSeNPs concurrently with D-gal in the same previous methods and doses. Blood samples were collected by cardiac puncture technique from anesthetized rats, then serum were obtained for measuring the following: Lipid profile including Serum concentration of Total Cholesterol using TC kit (Biosystem Spain), according to (**33**); Triglyceride utilizing Triglyceride kit (Biosystem .Spain), according to (**34**); low density lipoprotein-cholesterol and Very-low density lipoprotein-cholesterol depending on Friedewald formula(**35**) and High density lipoprotein-cholesterol by utilizing HDL-c kit (Biosystem . Spain), according to **(36**). Besides, blood sample were also obtained for measuring Total antioxidant capacity Kit (Elabscience, USA) and serum MDA by using Thiobarbituric acid (TBA) according to (**37).** Parts of the liver tissues were removed to detection of Gene expression of glutathione peroxidase (GSH-Px) using RNAzol® (Bioneer, korea) was used to extract total RNA from liver tissue, forward primer(5□AGT TCG GAC ATC AGG AGA ATG GCA□3) and Reverse (5□TCA CCA TTC ACC TCG CAC TTC TCA□3) primer used in quantification of gene expression using qRT-PCR techniques based SYBER Green DNA binding dye, and supported from (Bioneer, Korea) company, RT-PCR were identified according to criteria described by (**38)**.Statistical analysis: two-way analysis of various ANOVA and Least significant differences (LSD) test utilizing as prorated (**39**) at level of ($P<0.05$).

3-Results:

Green synthesis and characterization of Black currant selenium nanoparticles revealed appearance of brownish after 30 minute that changed gradually to reddish color and it becomes more stable (Figure 1). The optical absorbance of synthesized BcSeNPs was measured using UV-Vis spectroscopy. An absorption peak between (265-370 nm) confirms the presence of BCSeNPs (Figure 2). The pattern of SEM showed spherical shape nanoparticles with a diameter range of

18-50 nm in electron microscope (Figure 3). According to the result of XRD analysis in a current study the physical characteristic of particles in prepared compound is spherical and crystalized nanoparticles and the size of crystal was in range of 18 to 50 nm (Figure 4). Different distinct peak observed in Figure (5) in FTIR analysis, indicated the different functional group present in BCSeNPs. The distinct peak of BCSeNPs was seen at 3352.39 cm^{-1} correspond to OH: NH due to stretch vibration in amide A. Absorption peak at 2931.90 cm^{-1} correspond to C-H in $-CH_2$ in aliphatic compounds. While, the band at 1608.69cm^{-1} indicating NH_2 in primary amides. The peak at 1514.17 cm^{-1} is due to NH in secondary amides (amide II). The peak at 1359.86 cm^{-1} attributed to the C-H bending in alkanes. While, 1066.67 and 1035.81 cm^{-1} confirm C–O, C–C stretching vibrations, C–O–H, C–O–C bending vibrations in polysaccharides, protein and polyesters. C–X stretching in alkyl halides causes a band at 871.85 and 835.21 cm^{-1}. The band at 590.24 and 547.80 cm^{-1} is the result of C–N–C bending in amines

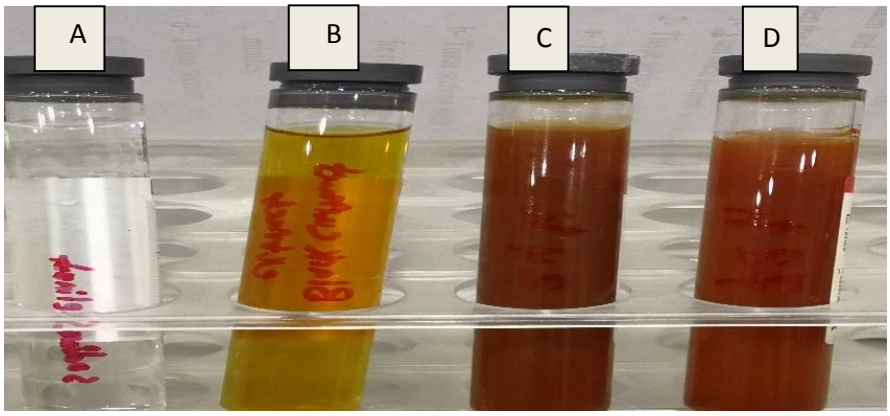


Figure 1: Image showed changing in color after the reduction of sodium selenite to BcSeNPs by black currant extract**. A:** Image showed sodium selenite solution, **B:** Image showed Black currant aqueous extract, **C**: Image showed Black currant selenium nanoparticles, D: Image showed BCSeNPs after 48-72 hour.

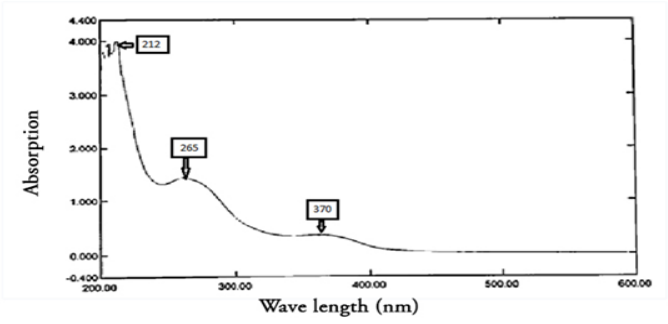


Figure 2. UV-Vis spectroscopy absorbance of selenium nanoparticles, making under carrying out sodium selenite with black currant extract in PH 9.

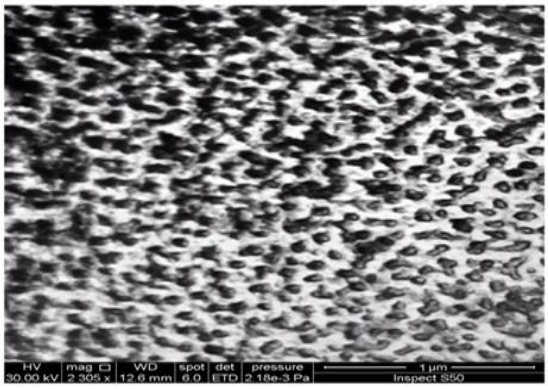


Figure 3. SEM test image of the selenium nanoparticles, making under carrying out sodium selenite with black currant extract in percentage 1:2 v: v ratio in PH 9 (1µm)

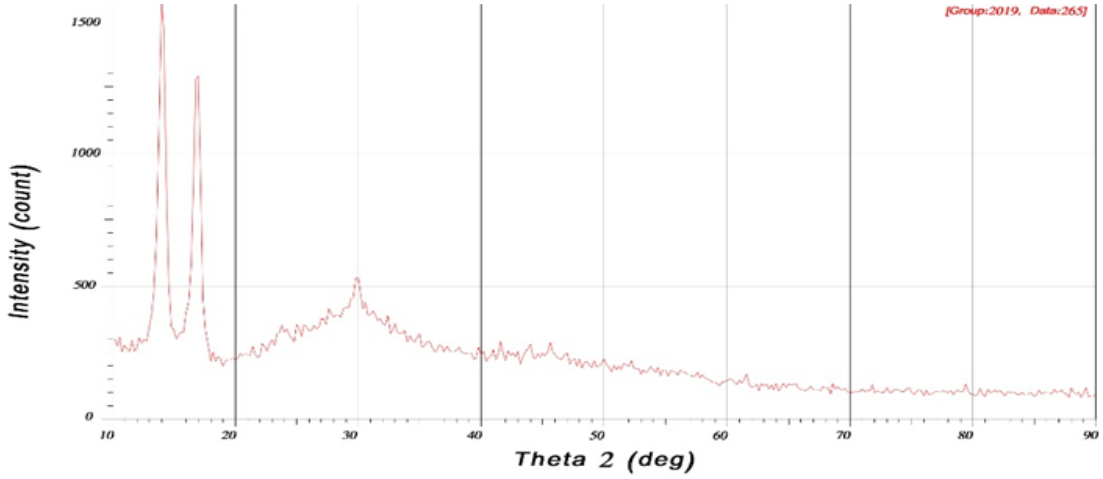


Figure 4: X-ray diffraction pattern for selenium nanoparticles, making under carrying out sodium selenite with black currant extract in PH 9.

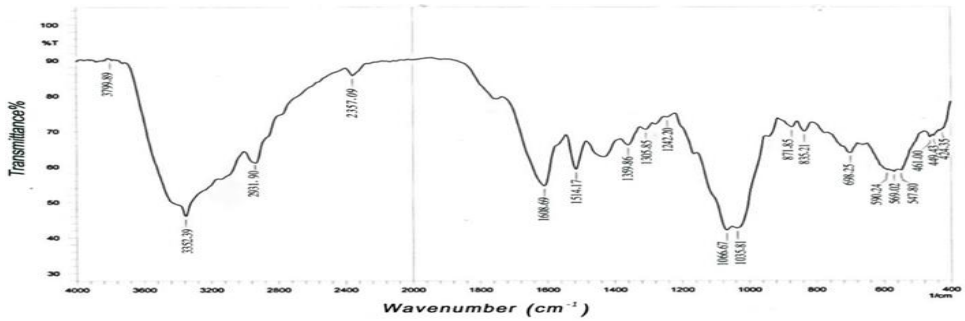


Figure 5: F-TIR spectroscopy for selenium nanoparticles, making under carrying out sodium selenite with black currant extract in PH 9.

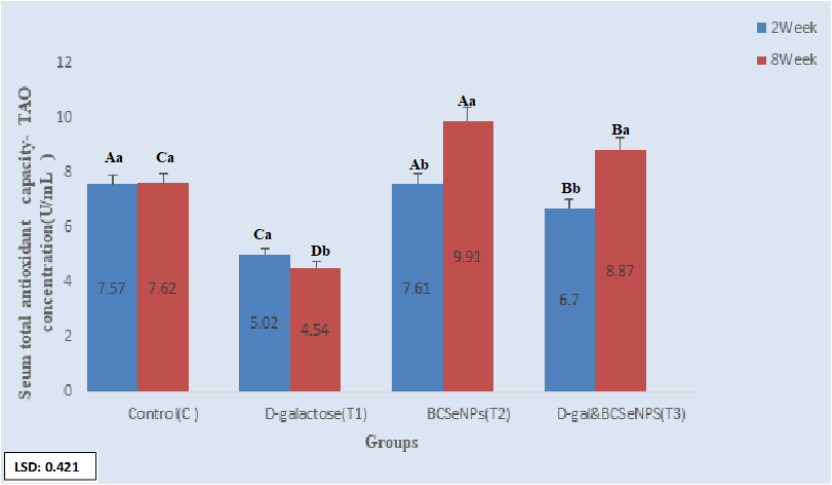
Antioxidant status:

At the end of the experiment significant increase ($p<0.05$) in serum TAO concentration was observed in BCSeNPs (T2) group comparing to the values in D-galactose (T1), D-gal& BCSeNPs (T3) and control (C) group (Histogram1). Within the time, significant ($P>0.05$) differences (increase or

decrease) where observation in all treated groups when compared to each other. Serum malondialdehyde (MDA) concentration (nmol/ml) showed significance elevation ($p<0.05$) in D-gal(T1) group comparing to the values in the control(C), T2 and T3 groups at the end of experiment. The result also showed that intubated black currant- selenium nanoparticles (BCSeNPs) 1mg/kg B.W. for 8 weeks caused significant decrease ($p<0.05$) in serum MDA concentration comparing to value in D-gal group (Histogram2).Within the time, D-galactose (T1) and D-gal &BCSeNPs (T3) groups were significant elevation in this parameter when compared to T2 and control groups. Results in histogram (3) indicated a significant ($p<0.05$) elevation in the fold change of GSH-Px gene expression levels in T2 and T3 groups when compared with T1 and control groups. There was non-significant decreasing the fold change in T 1 group (0.88 ± 0.24) as compared with control group.

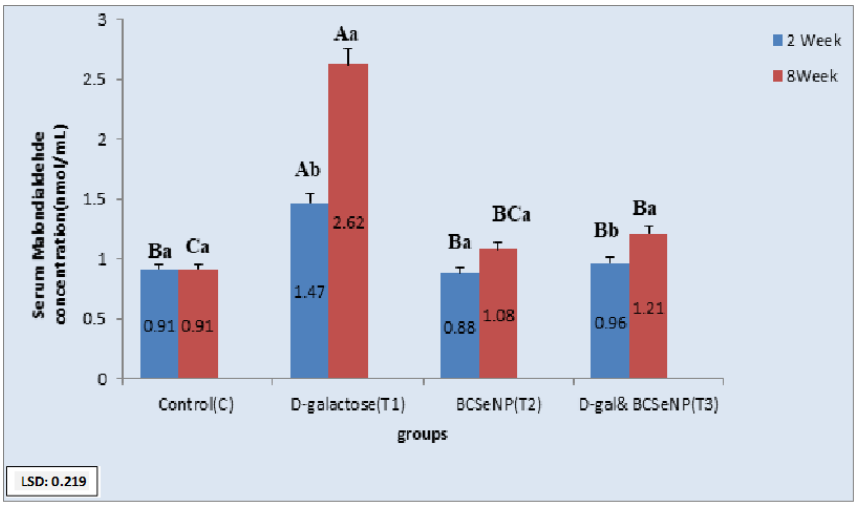
Lipid profile:

Comparing to control, T2 and T3 treated groups, at the end of the experiment, significant elevation ($p<0.05$) in serum (TC) was observed in T1 treated group that received 150 mg/kg B.W of D-galactose. The result also showed that BCSeNPs alone (groupT2), or their combination with D-galactose (groupT3), caused significant decrease in this parameter at week 2&8 comparing to the T1 treated group (Histogram4). Within the time, D-galactose(T1), BCSeNPs (T2) and D-gal &BCSeNPs(T3) groups showed significant elevation in this parameter at 8th comparing to 2nd weeks. Histogram (5) clarified the effect of BCSeNPs, D-galactose or their combination on serum TAG concentration in adult male rats. After 2 weeks, a significant ($P<0.05$) elevation in this parameter was observed in groups (T1) after *i.p.* of D-galactose (150 mg/kg B.W.) and T3 (combination of D-gal &BCSeNPs) comparing to the value in groups BCSeNPs and control (T2 and C). Further significant elevation ($P<0.05$) in serum TAG concentration was observed after eight weeks(T1) treated group comparing to values in other treated groups. In the same treated period, intubated black currant- selenium nanoparticles 1 mg/kg B.W. or T3 treated group caused significant decrease ($P<0.05$) in serum TAG concentration comparing to the value in T1 treated group.



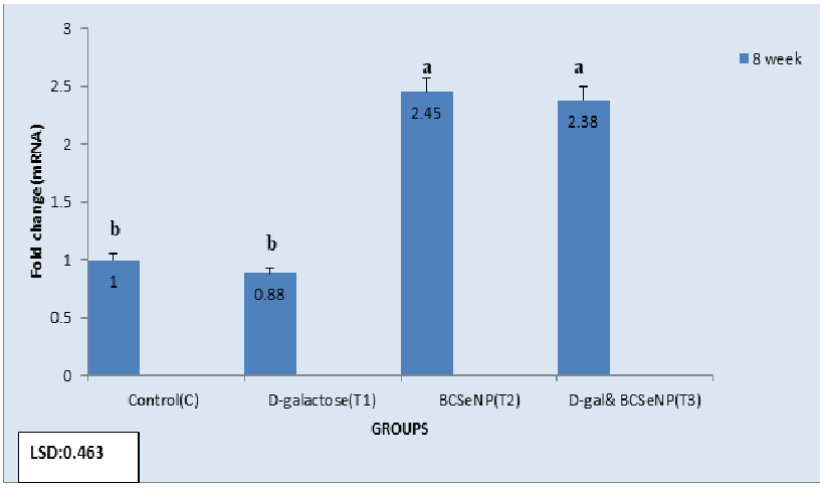
Histogram (1): Effect of D-galactose, Black currant selenium Nanoparticles (BCSeNPs) or their combination eight weeks on serum total antioxidant capacity-TAO concentration (U/mL) in adult rats.

Values are expressed as mean ± SE. n= 8. Various capital letters denote significant differences ($P<0.05$) between groups. Various small letters denote significant differences ($P<0.05$) between periods. Control (C): Intact rats where given daily normal saline (orally and *i.p.*). D-gal (T1): animals in this group were subjected to *i.p.* injection of D gal at a dose (150 mg/kg/day). BCSeNP (T2): rats were intubated black currant- selenium nanoparticles (1mg/Kg.B.W). D-gal& BCSeNP (T3): animals in this group were administered BCSeNPs concurrently with *i.p.* injected of D-gal.



Histogram (2): Effect of D-galactose, Black currant selenium Nanoparticles (BcSeNPs) or their combination eight weeks on serum malondialdehyde concentration (nmol/ml) in adult rats.

Values are expressed as mean ± SE. n= 8. Various capital letters denote significant differences (P<0.05) between groups. Various small letters denote significant differences (P<0.05) between periods. Control (C): Intact rats where given daily normal saline (orally and *i.p.*). D-gal (T1): animals in this group were subjected to *i.p.* injection of D gal at a dose (150 mg/kg/day). BCSeNP (T2): rats were intubated black currant- selenium nanoparticles (1mg/Kg.B.W). D-gal& BCSeNP (T3): animals in this group were administered BCSeNPs concurrently with *i.p.* injected of D-gal.

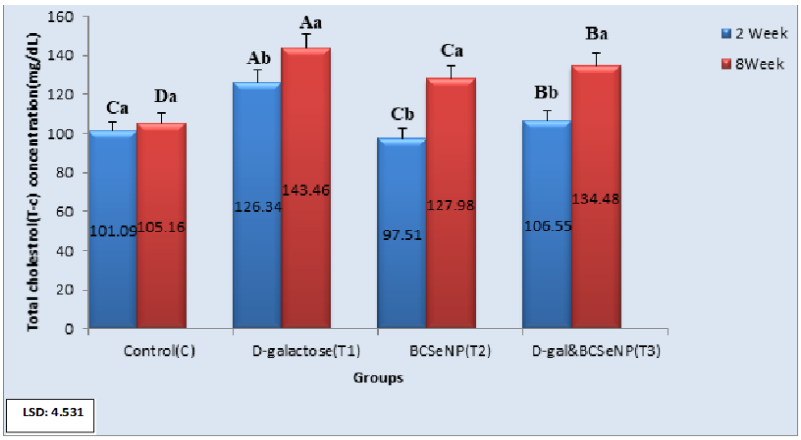


Histogram (3): Effect of D-galactose, Black currant selenium Nanoparticles (BCSeNPs) or their combination eight weeks on gene expression of glutathione peroxidase in adult rats.

Various small letters denote significant differences (P<0.05) between periods. Control (C): Intact rats where given daily normal saline (orally and *i.p.*). D-galactose (T1): animals in this group were subjected to *i.p.* injection of D gal at a dose (150 mg/kg/day). BCSeNP (T2): rats were intubated black currant- selenium nanoparticles (1mg/Kg.B.W). D-gal& BCSeNP (T3): animals in this group were administered BCSeNPs concurrently with IP injected of D-gal.

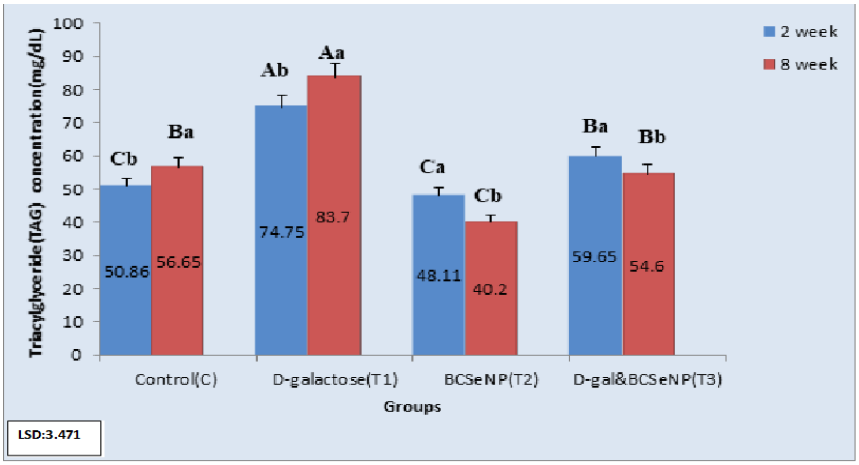
Besides, combination of D-gal &BCSeNPs caused significant (P<0.05) decrease in this parameter the value tends to normalize that of the control at the end of the experimental. At the end of the experiment, significant (p<0.05) elevation in serum HDL-c concentration was observed after intubated black currant- selenium nanoparticles (T2) or D-gal &BCSeNPs in group (T_3) comparing to the HDL-c value in D-gal treated group and control group (Histogram 6). Within the time, significant(p<0.05) increase in this parameter was observed in T1, T2 and T3 groups after eight weeks comparing to the value in two weeks. After two weeks, a significant (p<0.05) decrease in this parameter was observed in groups T2 under the effect of BCSeNPs and T3 groups after *i.p.* of D-galactose (150 mg/kg B.W.) & intubation BCSeNPs comparing to the V-LDL-c value in T1 treated groups which showed significant elevation. Further, significant elevation

(p<0.05) in serum V-LDL-c concentration was observed after eight weeks in D-galactose treated group (T1) comparing to values in other treated groups (Histogram 7). In the same treated period, intubated black currant-selenium nanoparticles (1mg/Kg.B.W) in T2 and T3 caused significant decrease (p<0.05) in serum V-LDL-c concentration comparing to the value in D- gal treated group. The result showed that intubated black currant- selenium nanoparticles (T2) groups caused significant decrease (p<0.05) in serum LDL-c concentration comparing to the value in other treated groups and the value becomes below that of the control at the end of the experimental. In comparison between periods, control and T3 groups showed no significant (P>0.05) difference between 2nd and 8th weeks periods, whereas T1 and T2 groups recorded significant (P<0.05) increase in the end of experiment (Histogram 8).



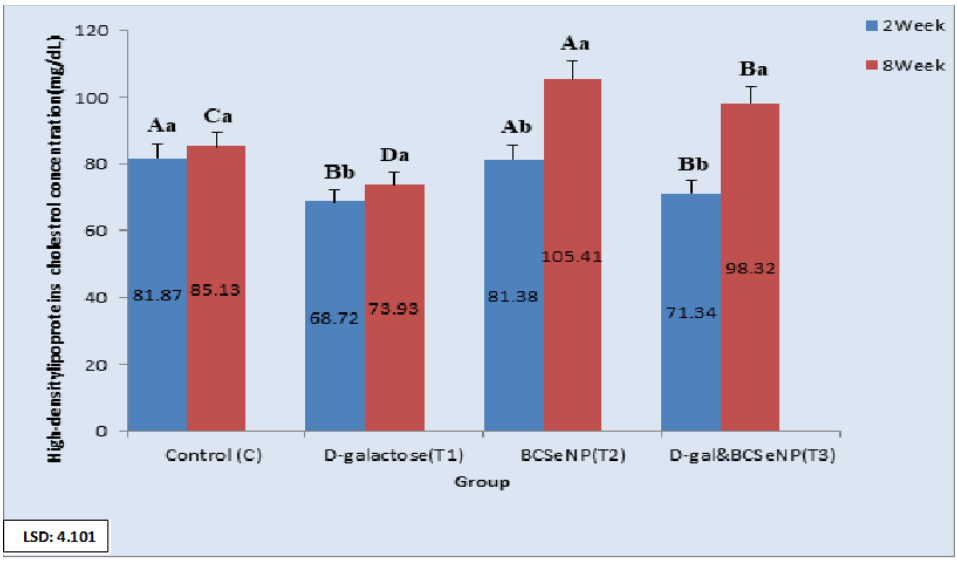
Histogram (4): Effect of D-galactose, Black currant selenium Nanoparticles (BCSeNPs) or their combination eight weeks on serum Total cholesterol concentration (mg/dl) in adult rats.

Values are expressed as mean ± SE. n= 8. Various capital letters denote significant differences (P<0.05) between groups. Various small letters denote significant differences (P<0.05) between periods. Control (C): Intact rats where given daily normal saline (orally and *i.p.*). D-gal (T1): animals in this group were subjected to *i.p.* injection of D gal at a dose (150 mg/kg/day). BCSeNP (T2): rats were intubated black currant- selenium nanoparticles (1mg/Kg.B.W). D-gal& BCSeNP (T3): animals in this group were administered BCSeNPs concurrently with *i.p.* injected of D-gal.



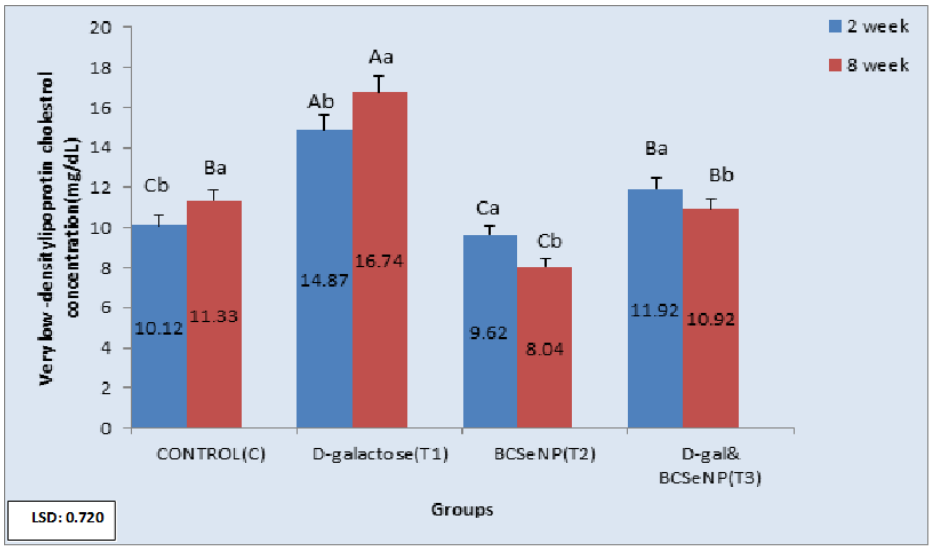
Histogram (5): Effect of D-galactose, Black currant selenium Nanoparticles (BCSeNPs) or their combination eight weeks on triglyceride (TAG) concentration (mg/dl) in adult rats.

Values are expressed as mean ± SE. n= 8. Various capital letters denote significant differences (P<0.05) between groups. Various small letters denote significant differences (P<0.05) between periods. Control (C): Intact rats where given daily normal saline (orally and *i.p.*). D-gal (T1): animals in this group were subjected to *i.p.* injection of D gal at a dose (150 mg/kg/day). BCSeNP (T2): rats were intubated black currant- selenium nanoparticles (1mg/Kg.B.W). D-gal& BCSeNP (T3): animals in this group were administered BCSeNPs concurrently with *i.p.* injected of D-gal.



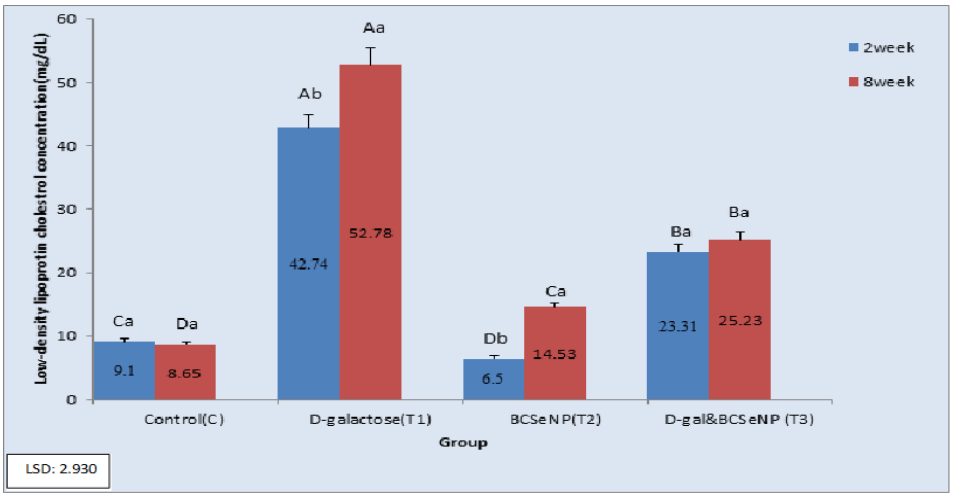
Histogram (6): Effect of D-galactose, Black currant selenium Nanoparticles (BCSeNPs) or their combination eight weeks on serum high-density lipoprotein cholesterol concentration (mg/dL) in adult rats.

Values are expressed as mean ± SE. n= 8. Various capital letters denote significant differences (P<0.05) between groups. Various small letters denote significant differences (P<0.05) between periods. Control (C): Intact rats where given daily normal saline (orally and *i.p.*). D-gal (T1): animals in this group were subjected to *i.p.* injection of D gal at a dose (150 mg/kg/day). BCSeNP (T2): rats were intubated black currant- selenium nanoparticles (1mg/Kg.B.W). D-gal& BCSeNP (T3): animals in this group were administered BCSeNPs concurrently with *i.p.* injected of D-gal.



Histogram (7): Effect of D-galactose, Black currant selenium Nanoparticles (BcSeNPs) or their combination eight weeks on serum very low-density lipoprotein cholesterol concentration (mg/dl) in adult rats.

Values are expressed as mean ± SE. n= 8. Various capital letters denote significant differences (P<0.05) between groups. Various small letters denote significant differences (P<0.05) between periods. Control (C): Intact rats where given daily normal saline (orally and *i.p.*). D-gal (T1): animals in this group were subjected to *i.p.* injection of D gal at a dose (150 mg/kg/day). BCSeNP (T2): rats were intubated black currant- selenium nanoparticles (1mg/Kg.B.W). D-gal& BCSeNP (T3): animals in this group were administered BCSeNPs concurrently with *i.p.* injected of D-gal.



Histogram (8): Effect of D-galactose, Black currant selenium Nanoparticles (BCSeNPs) or their combination eight weeks on serum low-density lipoprotein cholesterol concentration (mg/dL) in adult rats.

Values are expressed as mean ± SE. n= 8. Various capital letters denote significant differences (P<0.05) between groups. Various small letters denote significant differences (P<0.05) between periods. Control (C): Intact rats where given daily normal saline (orally and *i.p.*). D-gal (T1): animals in this group were subjected to *i.p.* injection of D gal at a dose (150 mg/kg/day). BCSeNP (T2): rats were intubated black currant- selenium nanoparticles (1mg/Kg.B.W). D-gal& BCSeNP (T3): animals in this group were administered BCSeNPs concurrently with *i.p.* injected of D-gal.

4-Discussion:

Antioxidant status

In current study significant elevation in serum TAO-C concentration, upregulation of GSH-Px gene expression with depression in MDA concentration was observed in BCSeNPs and T3 groups as compared to control and T1 treated groups, which refers to antioxidant effect of SeNPs (**9,40**). The positive effect of SeNPs could be related to the incorporation of selenium into proteins, such as selenocysteine (SeCys) and its preventive role in oxidative tissue damage (**19,41,42**). The best way to scavenge ROS by SeNPs could be through the ability to remove potential damage of lipid hydroperoxides and H_2O_2 via upregulation the levels of GSH-Px, SOD, and maintained GSH and productive mitochondrial function (**43,44**), indicating the antioxidant effect of SeNPs (**45,46**). Besides, the activity of superoxide dismutase, glutathione peroxidase and glutathione reductase in the serum and liver increased with selenium food supplementation (**47**). It has been observed that a mixture of selenium nanoparticles-grape seed extract (SeNPs - GSE) possesses antioxidant and anti-diabetic activities by decreasing oxidative stress and scavenger free radicals **(9,40).** Malondialdehyde is a measure of lipid peroxidation in the tissues, which considered as one of the important markers of oxidative stress that affect different organs (**48**). Its elevation by D-galactose in current study indicated oxidative damage induced by D-galactose. Several studies suggest a strong correlation between mitochondrial damage and ROS (mainly H_2O_2) production in cells **(49,50).** Such elevation in ROS could be accompanied with elevated MDA concentration and intense depletion in TAC (**51**), as recorded here in D-galactose treated group. Although D-galactose can be changed into glucose at normal concentrations. At high levels, D-galactose is oxidized into aldehydes and hydrogen peroxide, resulting in the generation of ROS and thus LPO product (**52**) including MDA. In agreement with our results, others observed that subcutaneous injection of D-galactose caused a significant decrease in antioxidant enzyme activities of CAT, GSH-Px, SOD, and T-AOC, as well as an increase in the MDA level **(53,54).**

lipid profile

Black currant selenium nanoparticles in here in study has been found to be inversely correlated to a case of dyslipidemia and positively correlated to HDL- cholesterol concentration. Similar to our results, in rats Se supplementation has been reported to increase low-density lipoprotein (LDL) receptor activity (**55**), lowered serum total cholesterol

(**56**). Alleviation of hyperlipidemic by SeNPs or selenium has been documented (**57-59**). On the contrary, Hunge and his worker **(60),** recorded that SeNPs supplementation is independently associated with dyslipidemia. The hypolipidemia induced by SeNPs could be through their efficacy in lowering gene expression of many enzymes associated with hepatic cholesterol metabolic especially HMG-COA reductase, cholesterol storage as well enhancing conversion of cholesterol to bile acids (**55, 59,61**). It is worth to mention that apart from its lipid lowering activity, selenium could also alleviate hyperlipidemia by reducing oxidative stress, through antioxidant seleno proteins enzyme especially GSH-peroxidase which play an important role in lipid metabolism (**59.61).** Similarly, present works showed a decrease in oxidative stress depression in MDA and elevation in TOA level and GSH-Px gene expression. Glucose and lipid metabolism disorder have been considered as important factor to D-galactose induced aging (**62**). Besides, D-galactose induced cardiac hypertrophy **(53)** has been reported. A substantial amount of evidence has demonstrated that ROS and AGEs, produced after high concentration of D-gal have been implicated in the pathological processes of age-related disease such as diabetes, arteriosclerosis (**63,64**) in which high lipid profile is regarded as major risk factor. Bo-Htay and his coworker (**65**) indicated that high concentration of D-gal aggravated cardiac disfunction and hyperlipidemia in rat with high fat diet. An elevation in mitochondrial ROS due to excess galactose caused generation of super oxide anion, decreased membrane potential, decrease lipid metabolism leading to dyslipidemia (**66-68**), where impairment of mitochondrial function, oxidative metabolism and depression in ATP generation (**69,70**) could occurred. Besides, oxidative stress induced by excess mitochondrial ROS, caused inhibition of insulin signaling and development of insulin resistance (**71**) that caused disturbance in lipid metabolism and dyslipidemia (**72,73**).

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