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Effect of selenium loaded *Ribes nigrum* nanoparticles on genetic markers in male rats with D- galactose induced toxicity.

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ABSTRACT

Jary Al-Kurdy MJ, Khudair KK., Effect of selenium loaded Ribes nigrum nanoparticles on genetic markers of male rats with D- galactose induced toxicity, Onl J Vet Res., 24 (5):312-327, 2020. Authors report effect of selenium nanoparticles loaded with black currant (*Ribes nigrum)* extract on serum caspase3, chromosomes, mitotic index, liver tunel cells and apoptosis in Wistar male rats given 150mg/kg D-galactose IP daily for 56 days. We generated 18-50nm spherical crystallite nanoparticles identified by UV spectroscopy, electron microscopy and X–ray diffraction. Groups of 8 rats each were then injected 150mg/kg D-galactose IP dissolved in 0.9% saline daily for 8 weeks (Group 1), gavaged 1mg/kg nanoparticles (2) or given both (3). Controls were given saline IP and orally (4). At days 14 and 56, cardiac blood was taken for caspase-3 and femur bone excised for chromosomal aberration and mitotic indices, and livers for DNA fragmentation and apoptosis. Compared with controls, we found significant ($P < 0.05$) declines in serum caspase (~-10 to -17%), and chromosomal acentric aberrations (~-4 fold) in rats given nanoparticles with or without galactose. In rats given only galactose we found large increases in serum caspase-A (~18%) chromosomal abberations (~12 fold) and mitotic indices (~34%). We found no tunnel liver cells in rats given selenium loaded *Ribes nigrum* nanoparticles with or without D galactose but present throughtout in those given only D-galactose. Results suggest that selenium loaded *Ribes nigrum* nanoparticles nanoparticles may reduce D- galactose genotoxicity in rats and exert anti-oxidants effects.

Key Words: Selenium, nanoparticles, black current, apoptosis, liver.

INTRODUCTION

Ahmad *et al.,* 2015; Peng *et al.,* 2016; El-Ghazaly *et al.,* 2017; Alagesan and Venugopal, 2019 and Menon *et al., (*2019) reported that selenium nanoparticles exhibited anti-mutagenic,

antibacterial, anti-inflammatory, anti-microbial and antioxidant activity. Hosnedlova *et al.,* 2018; Khurana *et al.,* (2019) found that oral nanoparticle formulations were well tolerated in patients. Selenium nanoparticles increased cytokines IFN-γ and IL-12 in splenocytes of tumor bearing mice and increased delayed hypersensitivity (Sarkar *et al.,* 2015). D-galactose is normally metabolized (Shahroudi *et al.,* 2017) but in excess converts to aldose and hydrogen peroxide by catalysis generating free radicals (Chen *et al*., 2018). Excess of D-galactose induced aging symptoms of cognitive dysfunction, oxidative stress but decreased antioxidant enzyme activity, immune response and mitochondria (Chang *et al*., 2016; Jeremy *et al*., 2017; Sulistyoningrum, 2017; Saleh *et al*., 2019). We describe effect of selenium nanoparticles loaded with black currant (*Ribes nigrum)* on chromosomes, mitotic index and apoptosis in Wistar male rats gavaged D-galactose daily for 56 days.

MATERIALS AND METHODS

Black currant aqueous extract was prepared as described by Gottimukkala *et al.,* (2017; Kaikai *et al.,* 2017*).* Characterization of nanoparticles were evaluated by Ultraviolet–visible spectroscopy (Metertech SP-8001 Taiwan) as described by (Mittal *et al*., 2013; Banerjee *et al.,* 2014), Fourier-transform infrared spectroscopy (FTIR) (Shimadzu Corporation Japan) (Mittal *et al.,* 2015; Rolim *et al.,* 2019), X-ray diffraction (XRD) (Shemadzu-6000 Japan) (Rietveld, 1969; Holzwarth and Gibson, 2011) and by scanning electron microscopy (SEM-Tescan Vega III, Czech) (Khoshnamvand *et al.,* 2018). After acclimatization for two weeks. thirty-two adult Wistar albino rats (aged 3 months and weighted 200±10g) were divided randomly and equally into four experimental groups (8/group), each were then injected 150mg/kg D-galactose IP dissolved in 0.9% saline daily for 8 weeks (Group 1), gavaged 1mg/kg nanoparticles (2) or given both (3). Controls were given saline IP and orally (4). Under anesthesia, cardiac blood was taken for serum caspase-3 (Rat CASP3 ELISA kit, Elabscience, USA). At sacrifice (day 56), femur bone marrow was excised to determine chromosomal aberrations as described by Savage, (1976) and mitotic index by Preston *et al.,* (1987). Liver tissue was stained for immunohistochemistry for apoptosis in cells (ApoBrdU-IHC DNA Fragmentation assay kit, Raybio, USA). One and Two-way ANOVA and Least significant differences (LSD) post hoc tests was used to determine significant ($P < 0.05$) differences between means (Snedecor George and Cochran, 1973).

RESULTS

Results are shown in Figures 1 to 18 and Tables 1 below.

Figure 1. Color reaction between *Ribes nigrum* extract and reduction of sodium selenite nanoparticles. A: sodium selenite, B: Black currant *Ribes nigrum* aqueous extract C: Black currant *Ribes nigrum* loaded selenium nanoparticles by 30min, D: by 48-72 hours.

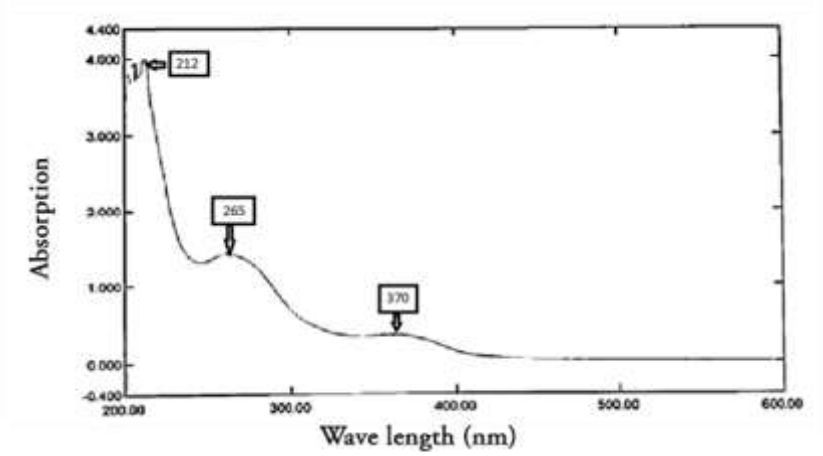

Figure 2: UV-Vis spectra absorbance of selenium loaded *Ribes nigrum* nanoparticles at pH 9.

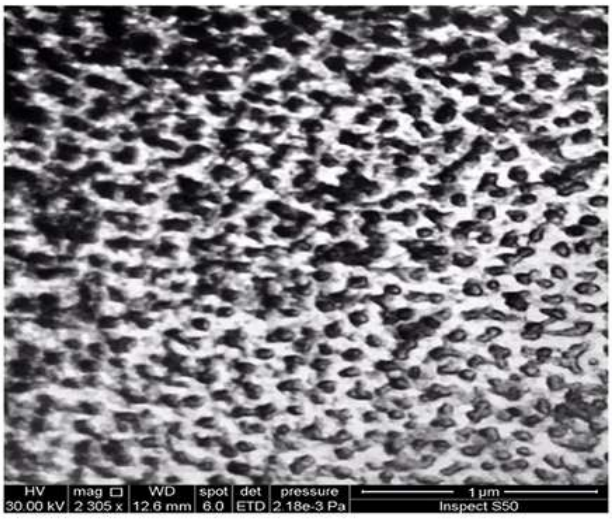

Figure 3: Electron microscopic image(1µm) of selenium loaded *Ribes nigrum* nanoparticles 1:2 ratio at pH 9

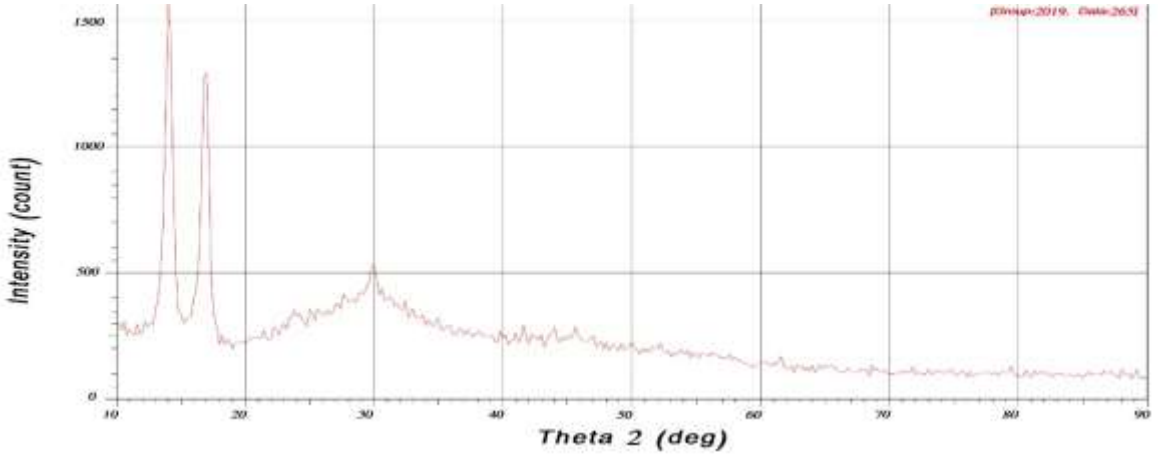

Figure 4. X-ray diffraction curve for selenium loaded *Ribes nigrum* nanoparticles at PH 9

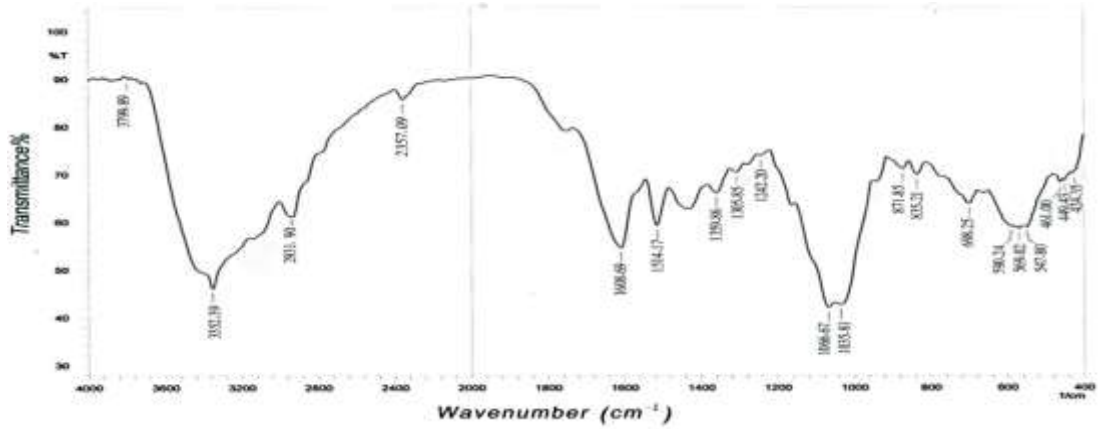

Figure 5. F-TIR spectroscopy of selenium loaded *Ribes nigrum* nanoparticles at PH 9.

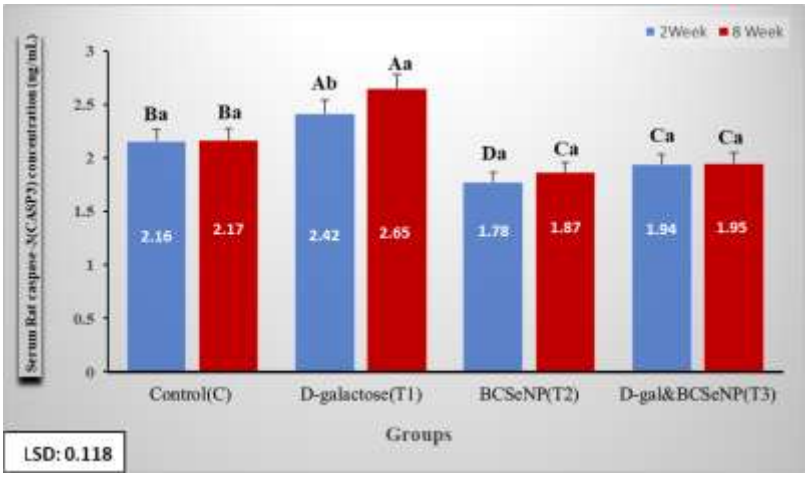

Figure 6. mean ± SE serum caspase-3 in rats (n=8) given 150mg/kg daily D galactose (T1) IP with (T3) or without (T2) 1mg/kg nanoparticles and saline controls (C) after 2 and 8 weeks. Different caps were significant between treatments, small letters between periods ($P < 0.05$).

Table 1 mean ± SE chromosomal aberrations (%) in rats (n=8) given 150mg/kg daily D-galactose IP with or without 1mg/kg selenium loaded *Ribes nigrum* nanoparticles and saline controls for 8 weeks.

	Chromosome aberrations %					
	Fragment	. Ring	Deletion	Polyploidy	Acentric	Total
Controls	0.03±0.002b	0.13±0.01b	0.10±0.004b	0.09±0.01 b	0.14±0.06a	0.51±0.07b
Galactose	0.23±0.03a 7*	2.48±0.16a 19*	0.23±0.01a 2*	3.10±0.28a 34*	0.14±0.01a	6.20±0.25a 12*
Particles	0.02±0.002b	0.12±0.005b	0.07±0.009b	0.09±0.01 b	0.03±0.006b - 4.6*	0.35±0.01b
Galactose + particles	0.02±0.002 b	0.08±0.01b	0.10±0.005b	0.13±0.01 b	0.04±0.008b - 4.2*	0.39±0.03b
$LSD_{0.05}$	0.045	0.246	0.032	0.413	0.102	0.391

Different letters (P>0.05) between treatments. *Xfold difference with controls

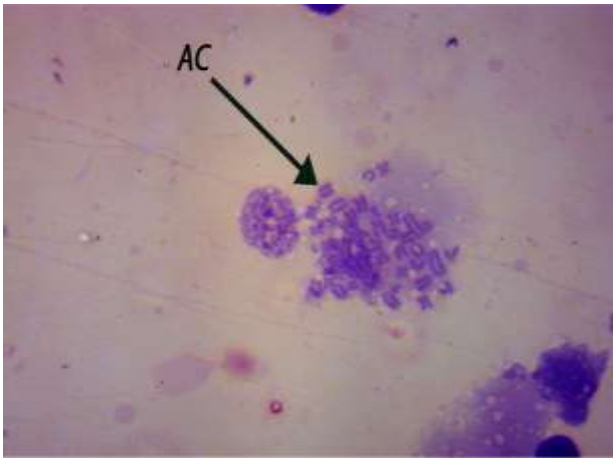

Figure 7: Bone marrow saline controls: Metaphase (AC) normal acentric chromosomes Giemsa stain(1000X)

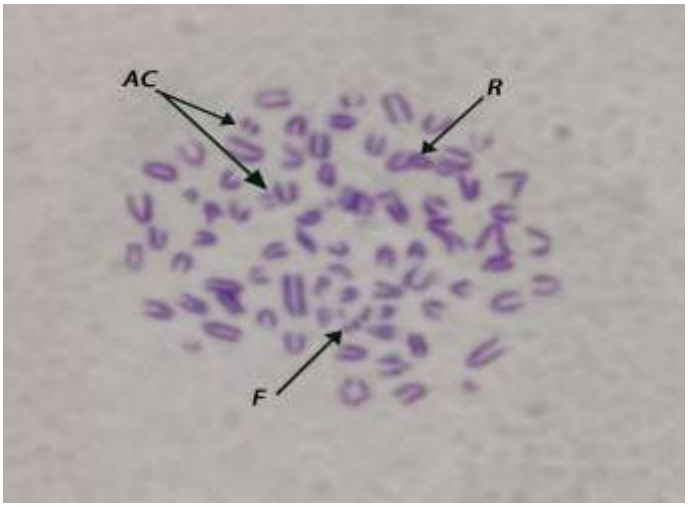

Figure 8. Bone marrow saline controls Metaphase (R) ring, (F) fragment, and (AC) acentric chromosomes. Giemsa stain (1000X).

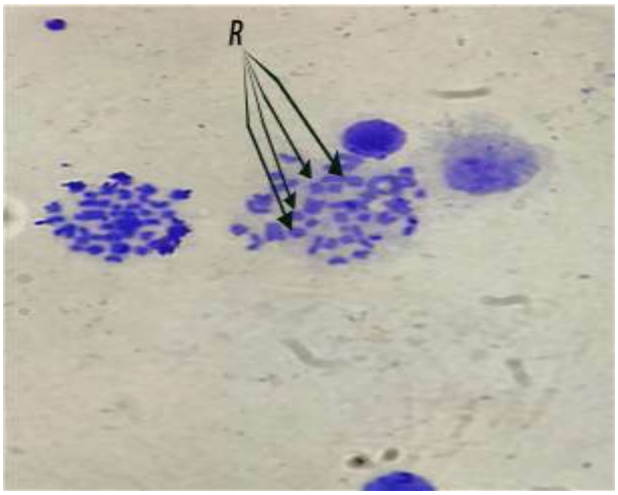

Figure 9. Bone marrow metaphase: rats given 150mg/kg D galactose daily for 56 days (R) ring chromosomes. Giemsa stain (1000X).

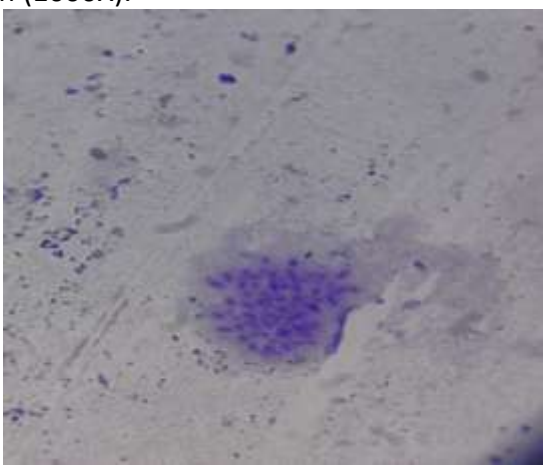

Figure 9. Bone marrow metaphase: rats gavaged 150mg/kg D galactose daily for 56 days showing Polyploidy in chromosomes. Giemsa stain(1000X).

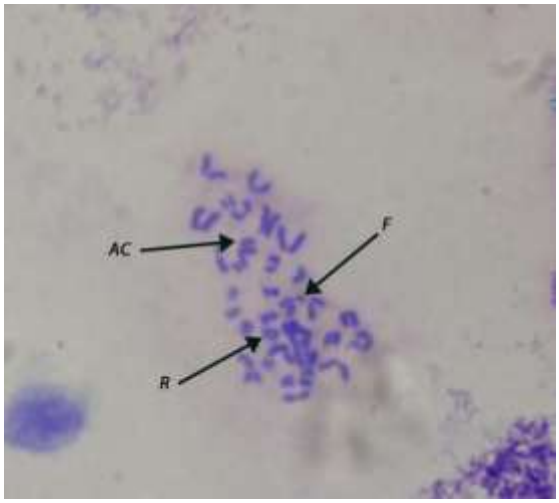

Figure 10. Bone marrow metaphase: rats given 1mg/kg selenium loaded *Ribes nigrum* nanoparticles IP daily for 56 days (F) fragmented, (AC) acentric and, (R) Ring chromosomes. Giemsa stain (1000X).

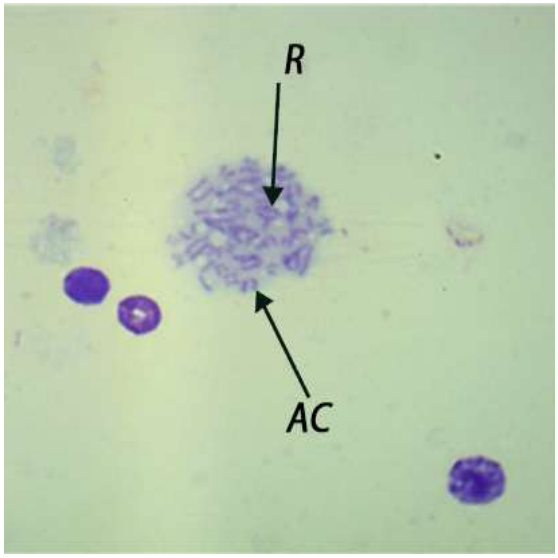

Figure 11. Bone marrow metaphase: rats given 1mg/kg selenium loaded *Ribes nigrum* nanoparticles IP daily for 56 day showing (AC) acentric and (R) Ring chromosomes. Giemsa stain (1000X)

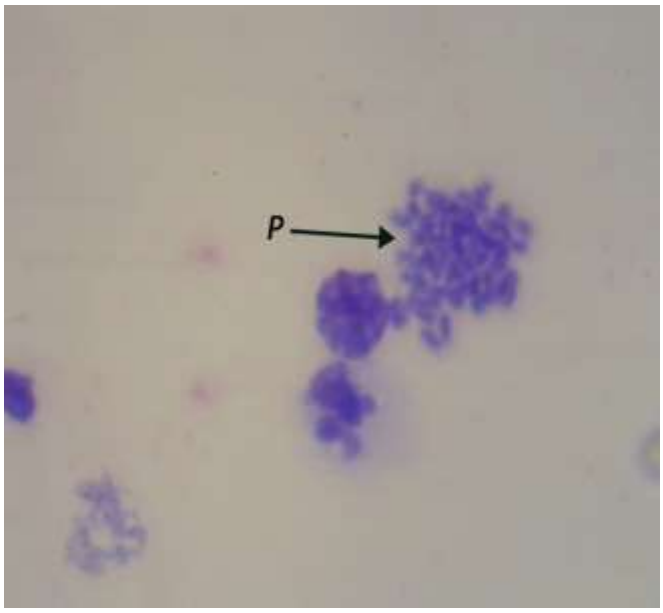

Figure 12. Bone marrow metaphase: rats given 1mg/kg selenium loaded *Ribes nigrum* nanoparticles IP with 150mg/kg D-galactose daily for 56 day showing polyploidy in chromosomes. Giemsa stain (1000X)

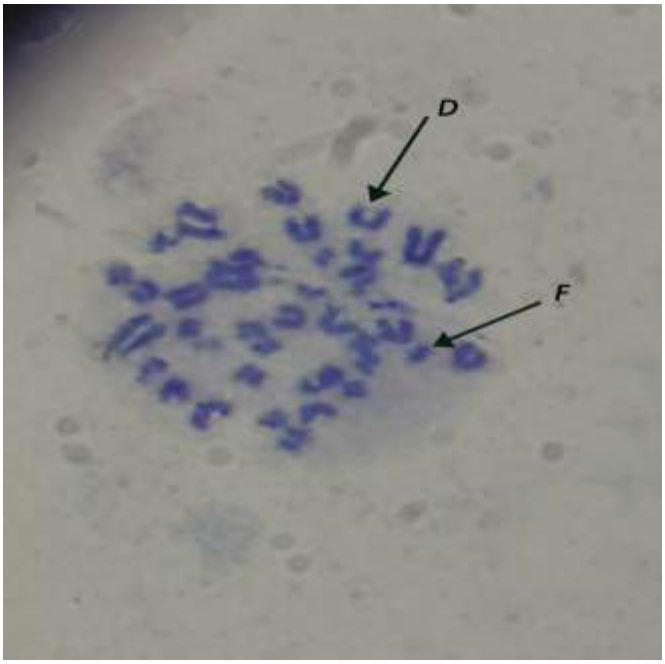

Figure 13. Bone marrow metaphase: rats given 1mg/kg selenium loaded *Ribes nigrum* nanoparticles IP with 150mg/kg gavaged D-galactose daily for 56 days showing (F) fragmentation, (D) deleted and polyploidy in chromosomes. Giemsa stain (1000X)

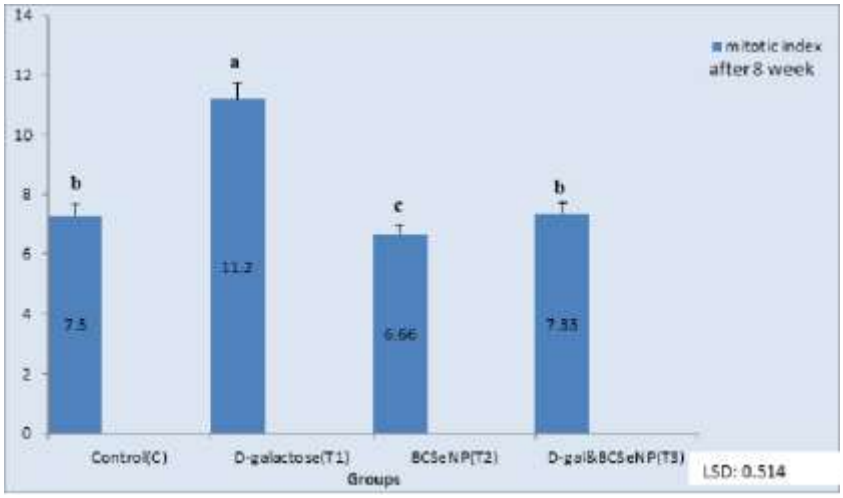

Figure 14: Mean ± SE mitotic indices in rats (n=8) given 150mg/kg daily D galactose (T1) IP with (T3) or without (T2) 1mg/kg nanoparticles and saline controls (C) after 2 and 8 weeks. Different letters between treatments ($P < 0.05$).

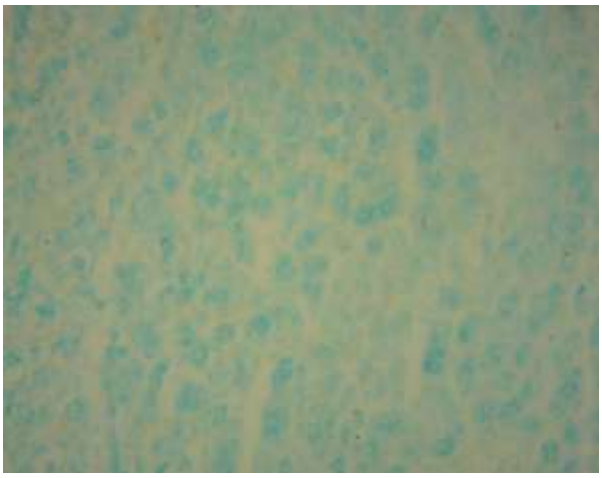

Figure 15. Liver saline controls shows no TUNEL cells determined by ApoBrdU-IHC DNA Fragmentation assay kit (400X).

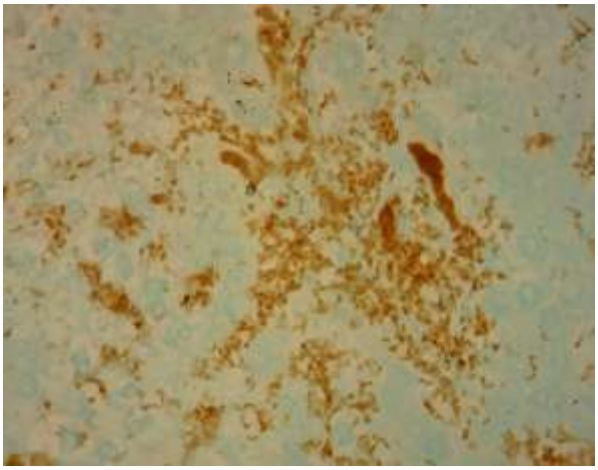

Figure 16. Liver of rats injected 150mg/kg D-galactose daily for 56 days reveals stained TUNEL cells throughout as determined by ApoBrdU-IHC DNA Fragmentation assay kit (400X).

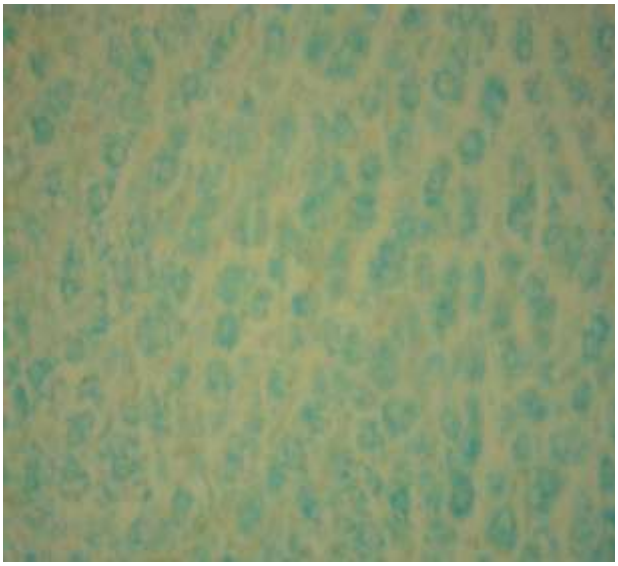

Figure 17. Liver rat injected 1mg/kg selenium loaded *Ribes nigrum* nanoparticles IP daily for 56 days shows no TUNEL cells determined by ApoBrdU-IHC DNA Fragmentation assay kit (400X).

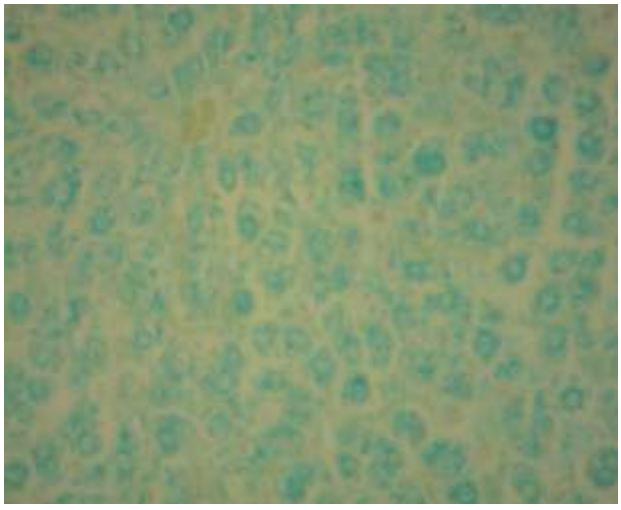

Figure 18. Liver rat injected 1mg/kg selenium loaded *Ribes nigrum* nanoparticles IP with 150mg/kg oral D-galactose daily for 56 days shows no TUNEL cells determined by ApoBrdU-IHC DNA Fragmentation assay kit (400X).

DISCUSSION

As per previous techniques (Sharma *et al.,* 2014; Gottimukkala *et al.,* 2017; Kaikai *et al.,* 2017) we found that the reduction reaction of *Ribes nigrum extract* with selenium nanoparticles occurred at ~30 minutes when the solution became red and was left to stabilize 48-72 hours as shown in Figure 1. We confirmed nanoparticles by UV-Vis spectroscopy with absorption peaks between (265-370 nm) as shown in Figure 2 (Fesharaki *et al.,* 2010; Zhang *et al.,* 2011; Harikrishnan *et al.,* 2012). Scanning electron microscopy revealed spherical amorphous crystalized nanoparticles with a diameter range of 18-50 nm (Figure 3) (Bunglavan *et al.,* 2014; Riva *et al.,*2018). X-ray diffraction revealed peaks at theta angle-2 with values of 24.142^{o}, 29.972^{o}, 41.91^{o}, 44.651^{o} and 46.782^{o} corresponded to hkl of (100), (101), (110),(102) and (111)

crystal planes (Figure 4) which corresponds to the reported value (JCPDS File No. 06-362) (Ingole *et al*.,2010; Sharma *et al.,* 2014; *Saratale et al.,* 2017). Figure (5) showed the F-TIR spectroscopy for selenium nanoparticles. The distinct peak of BCSeNPs was seen at 3352.39 cm-1 corresponds to OH: NH due to Stretch Vibration in Amide A. While absorption peak at 2931.90 cm-1 correspond to C-H in -CH2 in aliphatic compounds. While, the band at 1608.69cm-1 indicating NH2 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. However, the peaks at 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−1were due to C–N–C Bending in Amines (Kamnev *et al*.,2017; Tugarova *et al.,* 2018; Alagesan and Venugopal, 2019).

Compared with controls, we found significant ($P < 0.05$) declines in serum caspase (~-10 to -17%) in those given selenium nanoparticles with or without galactose (Figure 6) whereas in rats given only galactose, we found large increases (~18%). Zhu *et al.,* (2016) found that mRNA expression of p53, bax and caspase 3 declined in rats given cisplatin but increased following selenium treatment. Maiyo and Singh (2017) maintained that selenium could inhibit cancer by protecting DNA damage by free radicals and/or apoptosis. Excessive ROS by mitochondria can lead to apoptosis (Chen *et al*., 2018; park *et al*., 2019; kello *et al*., 2020) and activation of caspase-9 which activates caspases-3 and 7 (Kujoth *et al.,* 2005; Du *et al.,* 2019; Zhang *et al.,* 2019). D-galactose could inhibit expression of Bcl-xL an anti-apoptotic protein (Xia *et al*., 2019). These findings suggest that our selenium nanoparticles loaded with *Ribes nigrum* extract may have exerted anti-oxidant properties.

Compared with data from rats given only D galactose, we found significantly ($P < 0.05$) less chromosomal (-12-fold) and acentric aberrations (~-4 fold) as well as mitotic indices (~34%) in rats given nanoparticles with or without galactose (Figures 7-13). Microscopy revealed no TUNEL liver cells in rats given nanoparticles with or without D-galactose whereas in those given only galactose, we found TUNEL cells throughout Figures (15-18). Otton *et al.*, (2004) and Attia, (2010) reported that reactive oxygen species (ROS) can lead to apoptosis and chromosomal aberrations (Kubli and Gustafsson, 2012; Hausenloy and Yellon, 2013; Go *et al.,* 2014). Our results suggest that selenium nanoparticles loaded with *Ribes nigrum extract* may reduce D-galactose induced genotoxicity in rats.

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