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DP Patil
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

M Usharani
Professor and Head, Department of Veterinary Pharmacology, College of Veterinary Science, Mamnoor, Warangal, Telangana, India

Gopala Reddy A
Controller of Examination/Professor Veterinary Pharmacology, PVNRTVU, Hyderabad, Telangana, India

B Kalakumar
Professor, Veterinary Pharmacology, College of Veterinary Science, Hyderabad Telangana, India

GK Sawale
Assistant Professor, Department of Veterinary Pathology, Mumbai Veterinary College, Parel, Mumbai, MAFSU, Maharashtra, India

SV Londhe
Assistant Professor, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

SN Rindhe
Assistant Professor, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

Corresponding Author:
DP Patil
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Parbhani, MAFSU, Maharashtra, India

Subacute intravenous dose toxicity evaluation of nanoselenium particles in rabbits

DP Patil, M Usharani, Gopala Reddy A, B Kalakumar, GK Sawale, SV Londhe and SN Rindhe

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Abstract
A repeated dose toxicity study was conducted in male rabbits with administration of selenium nano-particles (SeNP) at weekly intervals by intravenous (I/V) route. Forty two male New Zealand White rabbits, were divided into four groups comprising six animals in each group. Group I served as normal control. SeNP were administered @ 0.1, 0.3 and 1mg/kg body weight to groups II, III and IV by intravenous administration weekly once for 28 days. The results revealed that SeNP administered in groups II to IV showed mild reduction in normal activity, ruffled fur, increase in sero-biochemical parameters like ALT, ALP, BUN, CPK, LDH, cholesterol and glucose. All the effects seen were significant (p<0.05) compared to the control group I. Altered histoarchitecture was observed in liver and kidney.

Keywords: Subacute toxicity, nanoselenium particles, intravenous route, rabbit

Introduction
Selenium (Se), originates from the Greek word "Selene" which refers the moon goddess. It was discovered by Jacob Berzelium in 1818. Selenium is a micronutrient metalloid of at least 25 human selenoprotiens and enzymes all containing selenocysteine with broad functions in biological system, including antioxidant properties, immune modulation, cancer amelioration and antiviral activities (Shakibaie *et al*., 2013) [13]. SeNP are envisaged widely in biomedicine due to their high bioavailability and diverse biological activities (Bhattacharje *et al*., 2017) [5]. It has been considered as a controversial nutrient because there is a very thin border between the lowest acceptable levels of intake and toxicity. (Aparna and Karunakaran 2016) [2] reported that selenium nanoparticles (SeNP) at the level of 0.225mg/kg in the diet of broiler chickens, destroys the cell membrane integrity due to oxidative stress resulting in low level of antioxidant status in broiler chicken and when given @ 0.187 mg/kg in diet improved oxidative resistance.
European Medicines Evaluation Agency (EMEA) [8] presented safety and ethical considerations for nanotechnology as "Nanotechnology task force reports" (EMEA 2007) and nanotechnology based medicinal products for human use (EMEA 2006) and in the same lines World Health Organization (WHO 1984, 1996) [16-17] Food and Drug Administration (FDA 1996) realized its safety evaluation, Scientists are assessing the appropriateness of existing methodologies to assess the potential risks of nanomaterials (Thomas *et al.*, 2006) [14]. Safety of occupational or accidental exposure of SeNP needs to be evaluated.

Materials and Methods
SeNP were synthesized using Sigma Aldrich USA chemicals in Department of Veterinary Pharmacology, College of Veterinary Science, Hyderabad, PVNRTVU, Telangana.

Experimental animals
All the rabbits were housed in clean steel cages in acclimatized room of laboratory animal house as per the specifications of Committee for the Purpose of Control and Supervision of

Experiment on Animals (CPCSEA) and given *ad libitum* feed and water throughout the experimental period. Acclimatization period of two weeks was observed before the start of the experiment. The experimental protocol was conducted with the approval from the Institutional Animal Ethics Committee (IAEC No. I -2008/C.V.Sc-IAEC, Hyderabad, Dt: 16/7/2018).

Animal Feed

Animal feed is prepared as per the standard formulation taken from Department of Animal Nutrition, College of Veterinary Science, Hyderabad.

Table 1: Experimental groups and their treatments (n=6).

Group	Treatments
I	Normal control (Greens and concentrate feed ad libitum)
II	SeNP @ 0.1 mg/kg body weight I/V, once weekly for 28 days.
III	SeNP @ 0.3 mg/kg body weight I/V, once weekly for 28 days.
IV	SeNP @ 1.0 mg/kg body weight I/V, once weekly for 28 days.

Statistical analysis

The analysis of variance of the data obtained was done by using independent sample T-test as per the methods given by (Panse and Sukhatme 1967) [11].

Results and Discussion

There were no behavioral and treatment related adverse reactions in control group rabbits throughout the experimental period. All the treatment group rabbits showed more activeness upto first week followed by slight sluggishness with ruffled fur and decrease in body weight.

TEC showed significant reduction on 14th day ($p<0.05$) in treatment groups compared to group I and nonsignificant increase in RBC count on day 28th except in group II ($p<0.05$) suggesting synergistic action on tissue repair and deceleration of disease progression indicating significant recovery from the pathology progress at the end of the study. Similar observations were also reported by Zhang and Julien (2011) [18].

Groups II, III and IV showed significant ($p<0.05$) reduction in TLC compared to other groups on day 14th. On day 28th, group III and IV showed significant ($p<0.05$) reduction in TLC compared to all other groups. Similar observations were reported by Benko *et al*. (2012) [4] where a dose dependent reduction in TLC was noticed.

On 28th day groups IV showed significantly ($p<0.05$) low haemoglobin concentration suggesting synergistic action on tissue repair and deceleration of disease progression indicating significant recovery from the anaemic condition. The PCV (%) concentration was reduced on day 14th significantly ($p<0.05$) in groups III, IV compared to other groups. On day 28th PCV concentration was increased significantly ($p<0.05$) in all groups compared to group I. Trung and Fotedar (2014) [15] reported reduced growth and PCV in juvenile yellow tail kingfish (*Seriola Plandi*) due to supra-nutritional selenium levels.

The activity of ALT, ALP and LDH in serum revealed a significant ($p<0.05$) increase in SeNPs treated rabbits as compared to control group on day 14th and 28th. Our findings are in agreement with those of Diskin *et al*. (1979) [7], who reported that liver is the main target organ of selenium toxicity and these findings are also in conformity with those of Zhang *et al*. (2008) [19].

The significantly ($p<0.05$) highest activity of CPK was found on 28th day with little less on 14th day and the significantly high BUN was found on 28th day with little increase on 14th day in all experimental groups compared to group I. Previous studies also demonstrated raised amount of serum urea after feeding rabbits with selenium supplemented forage (Saleh *et al*., 2015) [12] and feeding sodium selenite @ 2 mg/kg to 3 mg/kg orally in rabbits (Alam *et al*., 2016) [1].

The glucose concentration was increased significantly ($p<0.05$) on 28th day compared to 14th day in all treatment groups compared to group I. The hyperglycaemic response observed with SeNP indicated the role of supra-supplemented Se may be the probable risk factor for development of type-2 diabetes (Ogawa Wong *et al*., 2016) [10]. The significant increase ($p<0.05$) (of the levels of serum cholesterol on 14th and 28th day in treatment groups compared to group I. In the present study, significant ($p<0.05$) increase in the serum cholesterol level in treatment groups of rabbits is in agreement with the findings of Balogh *et al.*(2004) [3].

Histopathological studies from rabbits revealed dose-dependent pathological lesions in liver predominantly, and also in kidney. The principal histopathological lesions in rabbits were congestion in all the organs, hepatopathy, nephropathy and haemosiderin like pigment accumulation in liver tissue. Similar observations are noticed by Misra *et al* 2015 [9] and Davis *et al* 2012 [6].

Table 2: Effect on Total erythrocyte count of control and SeNP treated rabbits in various groups

Group	Treatment	Total erythrocyte count in µl (Mean ± SE)		
		0 day	14 day	28 day
I	Control	6.06±0.40^a	6.67±0.50^a	6.83±0.16^a
II	SeNP @ 0.1 mg/kg b.wt., I/V	6.08±0.50^a	5.05±0.42^b	7.46±0.10^b
III	SeNP @ 0.3 mg/kg b.wt., I/V	5.64±0.35^a	3.50±0.19^b	6.86±0.30^a
IV	SeNP @ 1 mg/kg b.wt.,I/V	6.04±0.51^a	4.07±0.49^b	6.41±0.42^a

Table 3: Effect on Total leukocyte concentration of control and SeNP treated rabbits in various groups

Group	Treatment	Total leukocyte concentration in U/l (Mean ± SE)		
		0 day	14 day	28 day
I	Control	11.33±0.54^a	13.00±1.30^a	10.01±1.33^a
II	SeNP @ 0.1 mg/kg b.wt., I/V	12.71±0.70^a	7.13±0.28^b	9.03±0.49^a
III	SeNP @ 0.3 mg/kg b.wt., I/V	12.36±0.61^a	7.96±1.13^b	8.73±0.51^b
IV	SeNP @ 1 mg/kg b.wt., I/V	12.58±0.79^a	9.18±0.43^b	7.83±0.47^b

Table 4: Effect on Hb (g%) of control and SeNP treated rabbits in various groups

Group	Treatment	Hb in g% (Mean ± SE)		
		0 day	14 day	28 day
I	Control	13.58±0.43[a]	14.23±0.58[a]	13.73±0.29[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	14.49±0.49[a]	17.52±0.38[a]	13.63±0.33[a]
III	SeNP @ 0.3 mg/kg b.wt., I/V	14.22±0.53[a]	17.65±0.48[a]	13.63±0.29[a]
IV	SeNP @ 1 mg/kg b.wt., I/V	15.65±0.38[a]	16.41±0.51[a]	12.00±0.65[b]

Table 5: Effect on PCV (%) of control and SeNP treated rabbits in various groups

Group	Treatment	PCV in % (Mean ± SE)		
		0 day	14 day	28 day
I	Control	38.90±1.80[a]	33.63±3.68[a]	51.33±3.15[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	37.95±1.29[a]	37.69±3.63[a]	44.95±1.10[b]
III	SeNP @ 0.3 mg/kg b.wt., I/V	42.13±2.13[a]	28.76±1.66[b]	46.87±1.14[b]
IV	SeNP @ 1 mg/kg b.wt., I/V	44.83±2.65[a]	25.36±0.36[b]	41.03±2.35[b]

Table 6: Effect on serum Creatinine phosphokinase level of control and SeNP treated rabbits in various groups

Group	Treatment	Serum Creatinine phosphokinase level in U/l (Mean ± SE)		
		0 day	14 day	28 day
I	Control	1.82 + 0.01[a]	1.91 + 0.09[a]	1.78 + 0.01[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	1.82 + 0.01[a]	1.95 + 0.04[b]	2.10 + 0.01[b]
III	SeNP @ 0.3 mg/kg b.wt., I/V	1.84 + 0.01[a]	1.97 + 0.05[b]	2.15 + 0.05[b]
IV	SeNP @ 1 mg/kg b.wt., I/V	1.80 + 0.02[a]	2.78 + 0.21[b]	2.87 + 0.23[b]

Table 7: Effect on serum BUN level of control and SeNP treated rabbits in various groups

Group	Treatment	Serum BUN level in mg /dl (Mean ± SE)		
		0 day	14 day	28 day
I	Control	21.68 + 0.29[a]	21.99 + 0.29[a]	21.65 + 0.28[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	21.33 + 0.32[a]	25.91 + 0.37[b]	24.95 + 0.37[b]
III	SeNP @ 0.3 mg/kg b.wt., I/V	21.74 + 0.56[a]	25.86 + 0.42[b]	24.15 + 0.48[b]
IV	SeNP @ 1 mg/kg b.wt., I/V	21.26 + 0.20[a]	26.97 + 0.29[b]	23.32 + 0.43[b]

Table 8: Effect on serum Alanine aminotransferase level of control and SeNP treated rabbits in various groups

Group	Treatment	Serum Alanine aminotransferase in (U/L) (Mean ± SE)		
		0 day	14 day	28 day
I	Control	94.06±0.74[a]	92.23±0.68[a]	94.03±0.69[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	95.79±1.11[a]	100.23±1.24[b]	106.23±1.48[b]
III	SeNP @ 0.3 mg/kg b.wt., I/V	93.66±0.89[a]	120.42±1.68[b]	133.08±1.75[b]
IV	SeNP @ 1 mg/kg b.wt., I/V	96.77±1.29[a]	123.71±1.50[b]	129.14±3.35[b]

Table 9: Effect on serum Alkaline phosphatase level of control and SeNP treated rabbits in various groups

Group	Treatment	Serum Alkaline phosphatase (U/L) (Mean ± SE)		
		0 day	14 day	28 day
I	Control	99.70±2.60[a]	98.76±0.57[a]	96.43±1.12[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	99.04±1.32[a]	109.76±1.65[a]	116.94±1.14[b]
III	SeNP @ 0.3 mg/kg b.wt., I/V	99.66±2.75[a]	125.14±1.35[b]	131.57±1.66[b]
IV	SeNP @ 1 mg/kg b.wt., I/V	98.81±2.78[a]	121.64±1.81[b]	143.38±7.20[b]

Table 10: Effect on serum Lactate dehydrogenase level of control and SeNP treated rabbits in various groups

Group	Treatment	Serum Lactate dehydrogenase (U/ml) (Mean ± SE)		
		0 day	14 day	28 day
I	Control	76.78±1.62[a]	77.39±1.75[a]	76.76±1.86[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	75.71±1.25[a]	82.46±0.83[b]	86.33±0.67[b]
III	SeNP @ 0.3 mg/kg b.wt., I/V	77.69±1.77[a]	83.61±2.15[b]	87.21±2.52[b]
IV	SeNP @ 1 mg/kg b.wt., I/V	75.19±1.62[a]	82.52±1.68[b]	85.89±2.09[b]

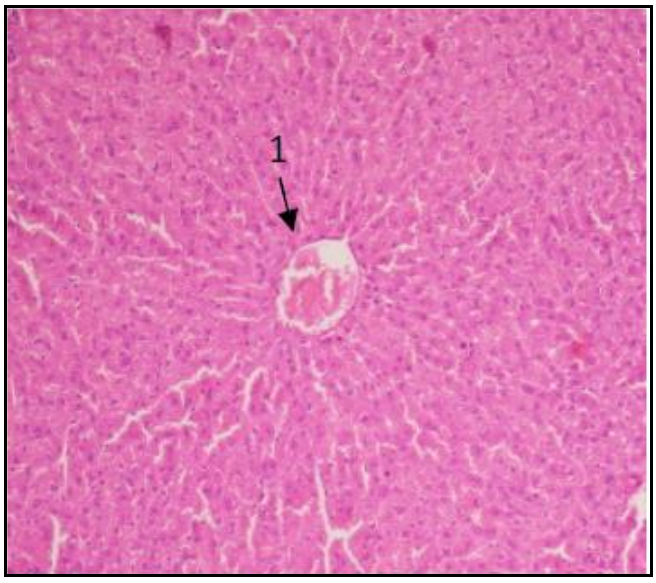
Table 11: Effect on serum Glucose level of control and SeNP treated rabbits in various groups.

Group	Treatment	Serum Glucose level in mg /dl (Mean ± SE)		
		0 day	14 day	28 day
I	Control	108.66±2.82[a]	104.83±2.70[a]	106.83±2.65[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	107.83±1.64[a]	112.16±1.27[b]	118.00±1.50[b]
III	SeNP @ 0.3 mg/kg b.wt., I/V	111.37±3.17[a]	114.66±3.07[b]	118.83±2.97[b]
IV	SeNP @ 1 mg/kg b.wt., I/V	112.33±1.38[a]	123.83±2.03[b]	131.66±1.80[b]

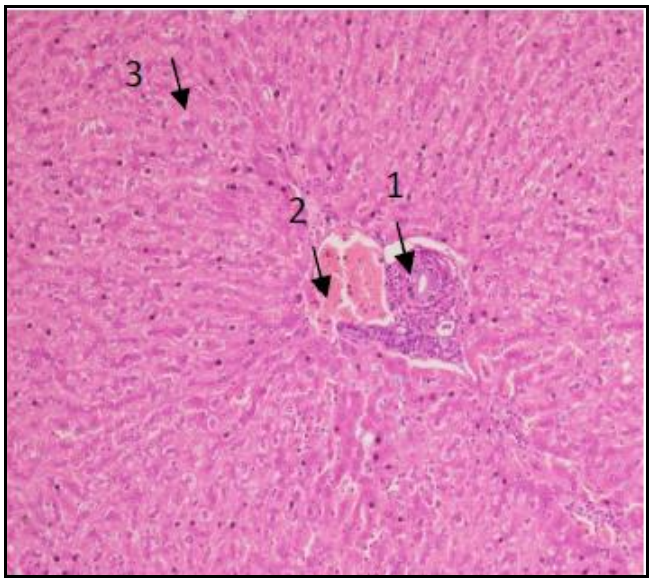
Table 12: Effect on serum cholesterol level of control and SeNP treated rabbits in various groups

Group	Treatment	Serum cholesterol level in mg /dl (Mean ± SE)		
		0 day	14 day	28 day
I	Control	46.00±1.75[a]	45.33±1.76[a]	45.33±1.83[a]
II	SeNP @ 0.1 mg/kg b.wt., I/V	44.33±1.11[a]	48.50±1.33[b]	47.00±1.34[b]
III	SeNP @ 0.3 mg/kg b.wt., I/V	44.50±1.60[a]	49.00±1.54[b]	49.00±1.59[b]
IV	SeNP @ 1 mg/kg b.wt., I/V	44.00±1.71[a]	50.33±1.87[b]	49.83±1.85[b]

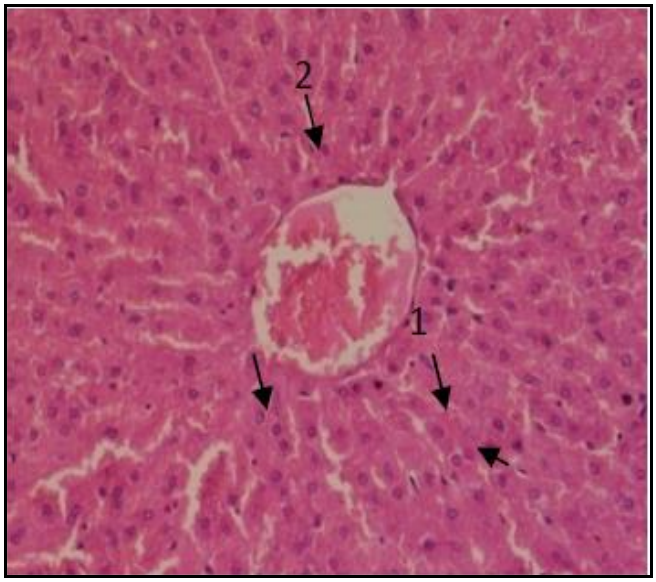
n=6 in each group.
Means bearing a, b superscripts within the columns are significantly different (p<0.05)



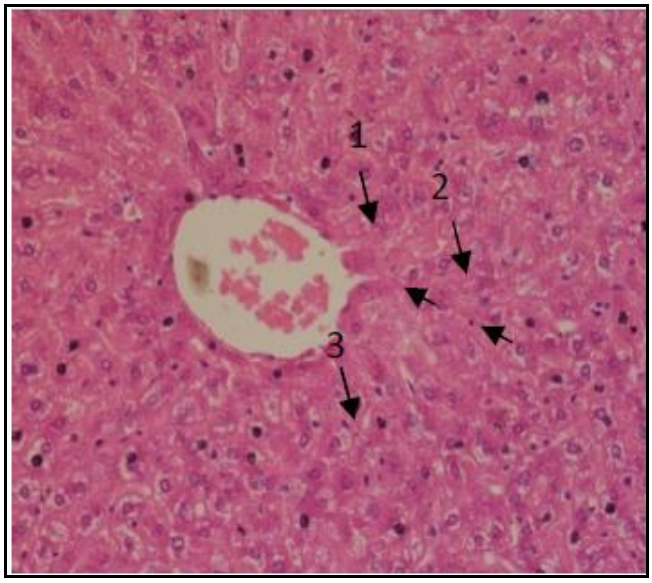
Photomicrograph of liver showing normal architecture with central vein (CV) (1) (Group I): H&E x 10



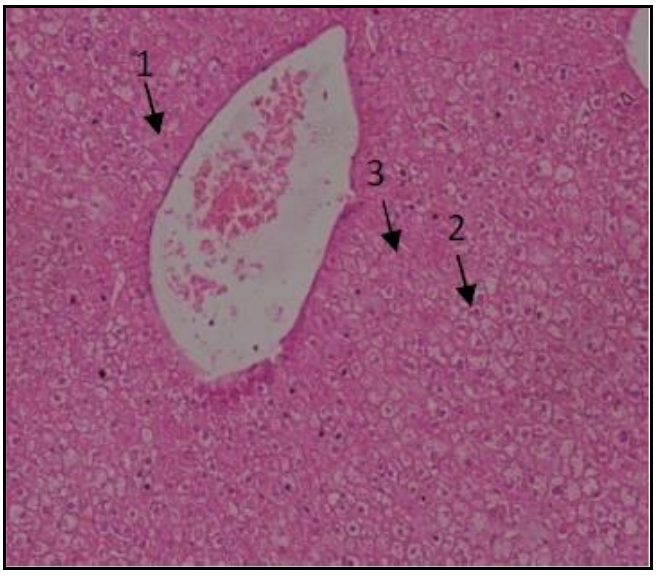
Photomicrograph of liver showing dilated venule thickened artery (1) shrunken bile duct, necrosed periportal hepatocytes with (2) haemosiderine like pigment(3) (Group II): H&E x 10



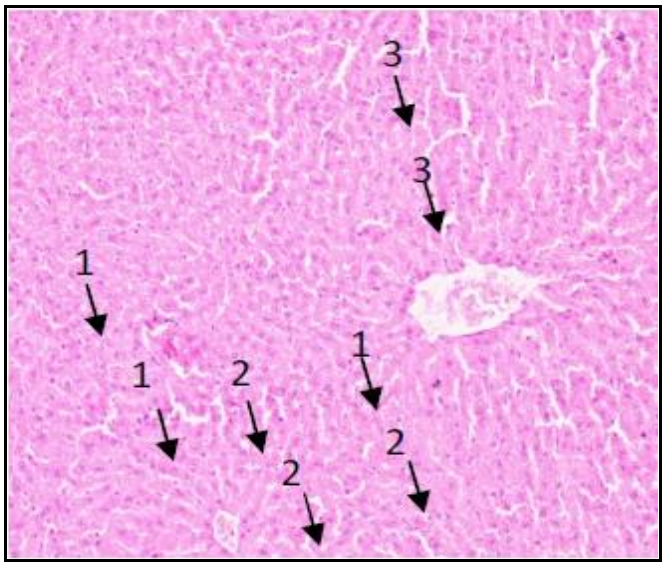
Photomicrograph of liver with normal nucleus central vein (1) and hepatic cords (2) (Group I): H&E x20



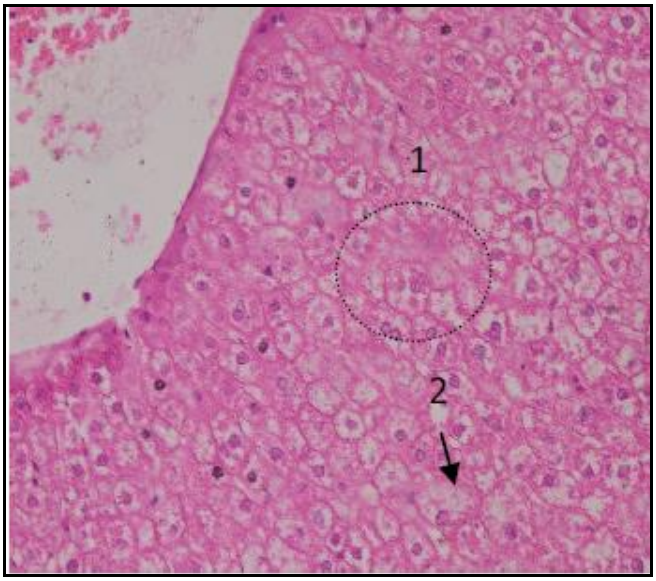
Photomicrograph of liver showing degenerated hepatocytes with swollen (1) and karryorrhexic nuclei with congestion (2) and haemosiderine like pigment(3) (Group II): H & E x 20



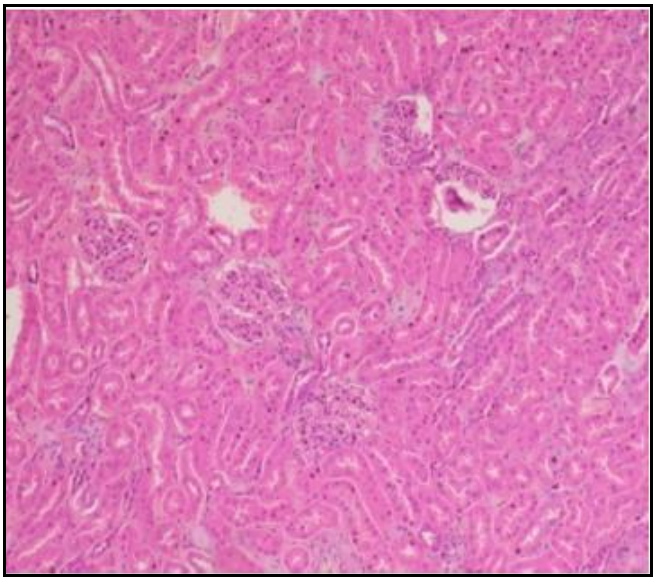
Photomicrograph of liver showing congestion of central vein (1), cloudy swelling to vacuolar degeneration of hepatocytes (2) with pyknosis (3) (Group III): H&Ex 10



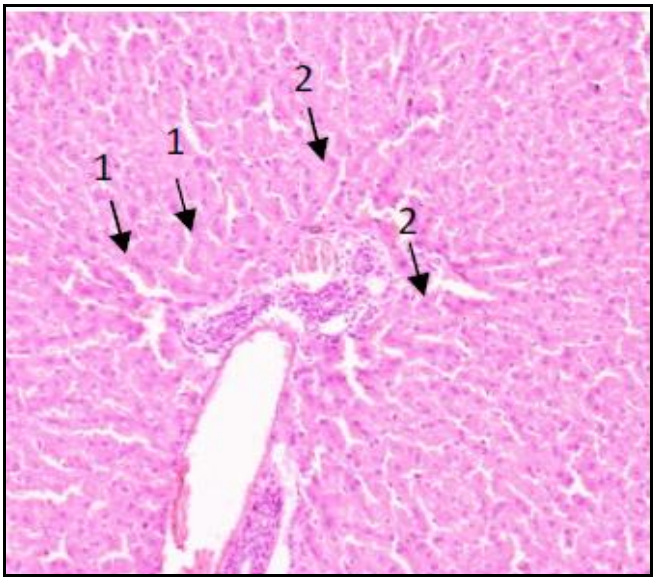
Photomicrograph of liver showing inflammatory cells infiltration in periportal area (1), pyknotic nuclei (2) and hepatocytes showing mild sinusoidal dilatation (3) (Group IV): H&F x 10



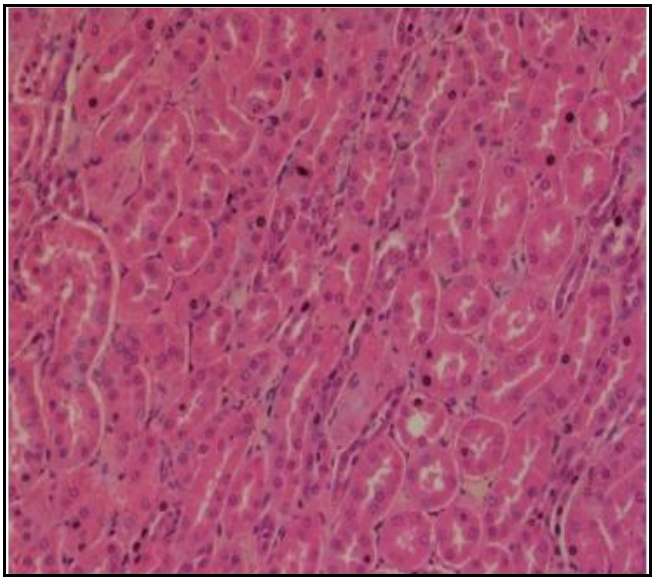
Photomicrograph of liver showing disrupted hepatic cord (1) with irregular granular cytosol of some cells (2) (Group III): H&E x 20



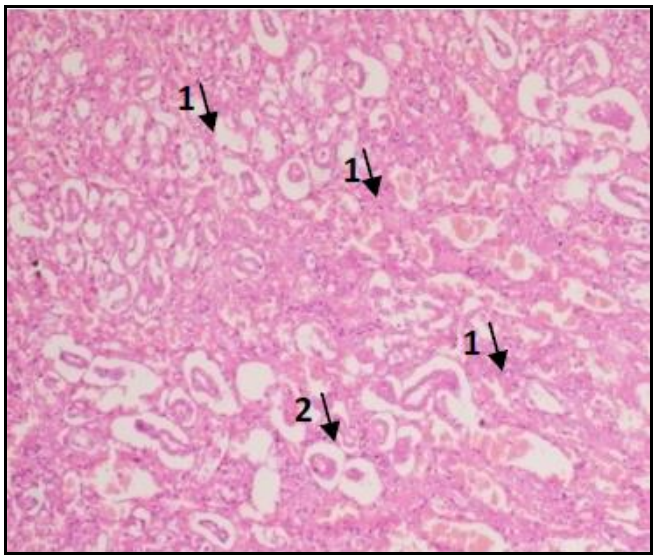
Photomicrograph of kidney showing normal architecture with normal and uniform nucleus (Group I): H&E x 10



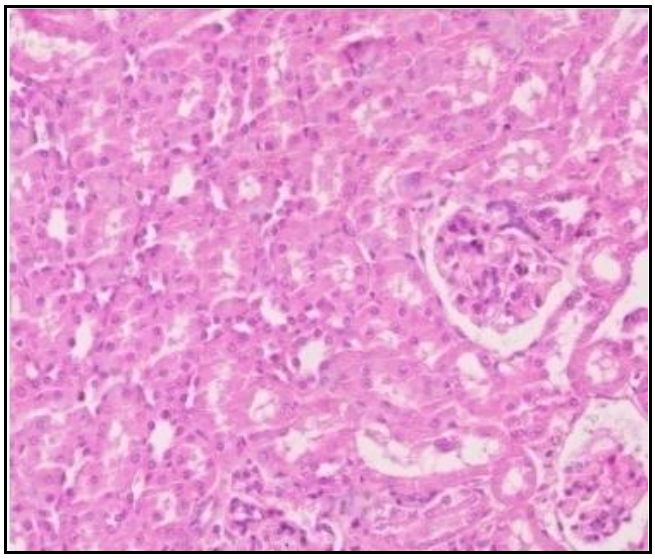
Photomicrograph of liver showing some necrosed hepatocytes (1), with mild dilatation of sinusoids (2) (Group IV) H&E x 10



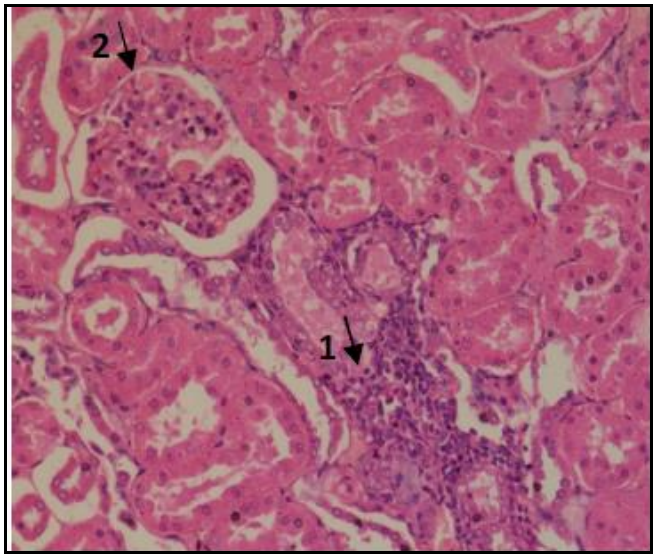
Photomicrograph of kidney showing normal tubular epithelium (Group I): H&E x 20



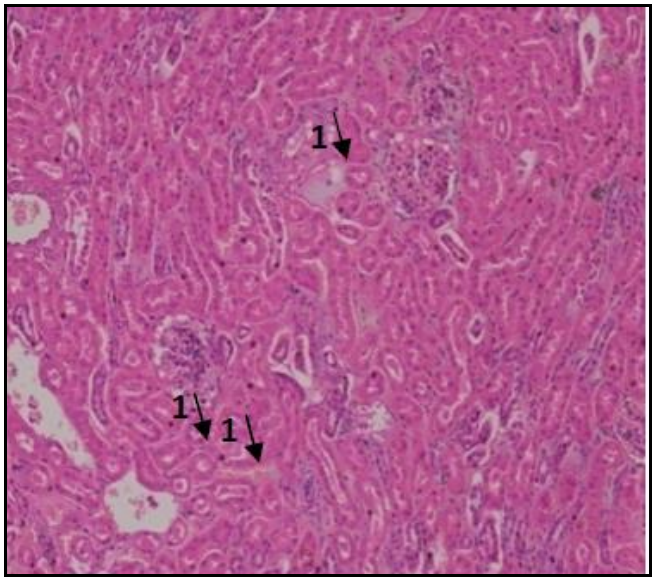
Photomicrograph of kidney showing degeneration, desquamation of epithelial cells and casts in tubules (10, cystic tubules (2) (Group II): H&E x 10



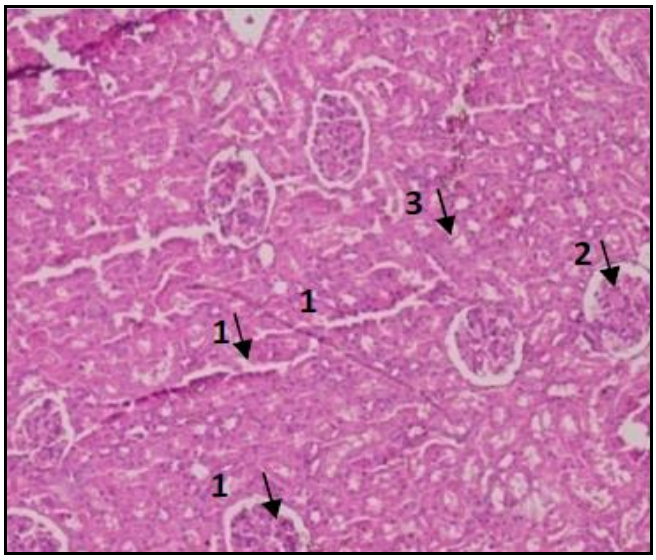
Photomicrograph of kidney showing distortion of epithelial cells, necrosis of focal areas of tubules (Group III): H&E x 20



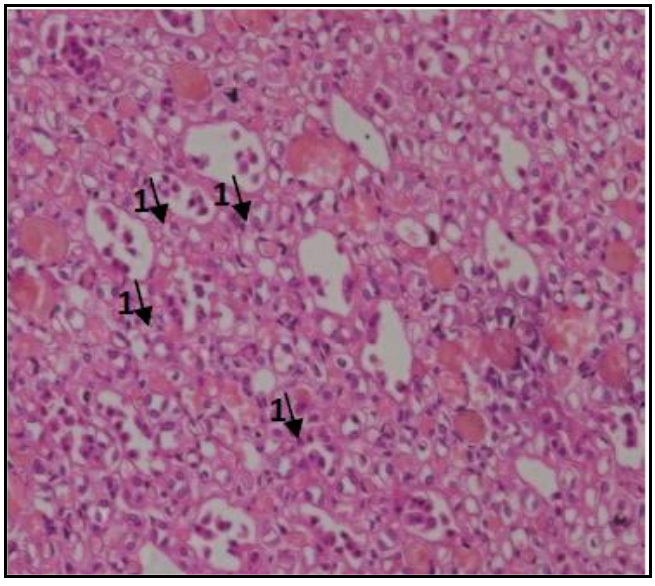
Photomicrograph of kidney showing focal areas of interstitial nephritis with haemorrhages and widening desquamated epithelium (1) and swollen glomeruli (2) (Group II): H&E x 10



Photomicrograph of kidney showing vacuolar degeneration (1) (Group IV): H&E x 10



Photomicrograph of kidney showing dilated tubules (1), shrunken to swollen glomeruli (2), pyknotic nuclei of tubule epithelial cells (3) (Group III): H&E x 10



Photomicrograph of kidney showing prominent vacuolar degeneration of tubules, congestion of blood vessels (1) (Group IV): H&E x 20

Conclusion

I/V administration of SeNPs showed mild, moderate and severe toxicity response to low, moderate and high dose of SeNPs respectively indicating lowest dose can be used for therapeutic purpose in cancer patients. The therapeutic use of nanomaterials in medicine requires a different framework which balances the therapeutic benefit against the potential risk of harm.

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