Toxicology Reports 5 (2018) 771–776

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Evaluation of selenium nanoparticles and doxorubicin effect against hepatocellular carcinoma rat model cytogenetic toxicity and DNA damage

Omaima M. Abd El-Moneim*, Abeer H. Abd El-Rahim, Naglaa A. Hafiz

Cell Biology Department, National Research Centre, El Tahrir Street, 12622, Dokki, Giza, Egypt

ARTICLE INFO

Keywords:
Hepatocellular carcinoma model
Selenium nanoparticle
Doxorubicin
Cytogenetic
DNA damage

ABSTRACT

The present study aimed to demonstrate the potent role of nanoselenium and Doxorubicin in retrogression of genotoxicity induced in hepatocellular carcinoma rat model by studying chromosomal aberration, micronuclei formation, DNA fragmentation as well as comet assay. Male rats hepatocellular carcinoma model were treated with Se-Nanoparticles, Doxurobicin (DOX) and the combination of both. The results revealed the protective effect of nanoselenium, Doxorubicin and their combination on bone marrow cytogenetic toxicity by decreasing chromosomal aberrations and micronuclei formation as well as their effects on rat's liver by decreasing DNA damage. Nevertheless, the treatment with nanoselenium either alone or in combination with Doxorubicin was more effective than treatment with doxorubicin alone.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancies worldwide; it is affecting approximately one million people every year [1]. The risk of HCC increased in chronic infection with hepatitis C virus (HCV), approximately 20% of patients infected with HCV have diseases progress to cirrhosis, and about 40% of them develop HCC after 10–15 years [2]. HCC is also arises due to inflammation that increase DNA damage and chromosomal aberrations [3], It is recognized that both genomic instability and genetic alteration are common features of human HCC.

Doxorubicin is the main drug for the treatment of HCC, but its effect is limited due to its resistance to cancer cells [4]. Until now, there is still no effective treatment for both intermediate and end-stage of hepatocellular carcinoma. Furthermore, anticancer substances which can overcome drug resistance and prevent hepatoma cell death in advanced liver cancer are urgently needed.

Recently, the combination of biotechnology and nanotechnology lead to development the cancer treatment and had become used in many applications such as molecular diagnosis, molecular imaging, and targeted therapy, and this open new prospects for cancer treatments [5–7]. The basic rule of nanomaterials is that, these materials have optical, structural, or magnetic properties which do not present in the molecules or bulk solids [8,9]. Selenium (Se) is a mineral trace element, which is very important to humans and animals and has a very important role in cancer cell, it acts as chemotherapeutic and chemopreventive agent has been demonstrated in many epidemiological, preclinical, and clinical studies [9–11]. Se nanoparticles (SeNPs) have attracted increasing attention in the past due to their antioxidant activities and low toxicity [12–14]. Several studies showed that Nano - Se has antioxidant activities in vitro and in vivo through the activation of selenoenzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) which prevents oxidative damage to body tissues [15,16].

Thus, the goal of this study was to clarify the potential role of the treatment of Selenium nanoparticles, Doxorubicin and their combination on chromosome aberrations, micronuclei formations and DNA damage induced in hepatocellular carcinoma rat model.

2. Materials and methods

2.1. Nano-selenium preparation (Nano-Se)

Nano-selenium was prepared by the method of Dwivedi et al. [17]. Briefly, in aqueous medium, sodium selenosulphate precursor was reacted with different organic acids under special conditions. Then, the synthesized nanoparticles (Nano-Se) 20–60 nm in size (Fig. 1) were separated using centrifuge in a high-speed and redispersed in aqueous medium with a sonicator.

* Corresponding author.
E-mail address: emy.monem@yahoo.com (O.M. Abd El-Moneim).

https://doi.org/10.1016/j.toxrep.2018.07.003
Received 13 February 2018; Received in revised form 29 June 2018; Accepted 24 July 2018
Available online 25 July 2018

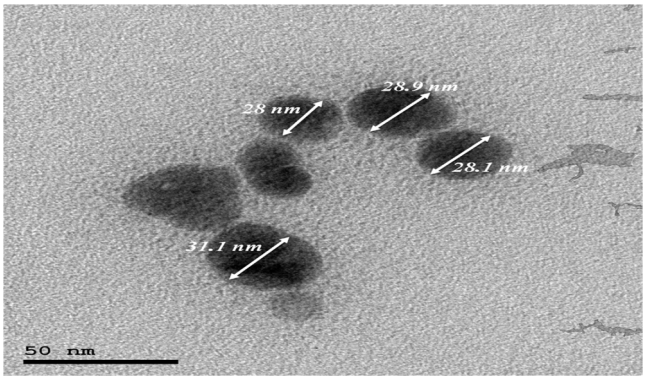

Fig. 1. High-resolution transmission electron microscopy image of nano-se.

2.2. Nano-Se characterization

Nano-Se was characterized by JEOL JEM-2100 high resolution transmission electron microscope (TEM) at an accelerating voltage of 200 kV.

2.3. Animals

Sixty adult male albino rats (140 ± 10 g) obtained from the Animal House of National Research Centre, Giza, Egypt, were used in the present study. The animals were housed in plastic cages with wood shavings at a freely ventilated and naturally illuminated room with controlled temperature (25 ± 5 °C) and humidity (50 ± 10%). Animals were fed with standard laboratory rat diet consisting of salts mixture 4%, casein 10%, corn oil 10%, vitamins mixture 1%, and cellulose 5% completed to 100 g with corn starch and water provided *ad libitum*. Animals were adapted to their environment for at least 10 days before the initiation of the experiment. The experimental protocol complied with the guidelines for animal's experiment which were approved by the Ethical Committee of the Medical Research of the National Research Centre.

2.4. Experimental design

After the acclimatization period, the rats were randomly assigned into six groups (10 rats each). Group (1) Healthy group set as negative control received 0.5 ml saline daily during the experimental period. Group (2) healthy group treated with nano-Se 5 mg/Kg/body weight three times/week according to Zhang et al. [13] (nano-se group). Group (3) set as hepatocellular carcinoma model received N-nitrosodiethylamine (NDEA) orally at a dose of 20 mg/Kg /body weight five times a week during a period of four weeks, then the animals received 10 mg/Kg/body weight for other one week [18]. Group (4) hepatocellular carcinoma model treated with nano-se with the same previous dose (nano-se treated group). Group (5) hepatocellular carcinoma model treated with doxorubicin (HCC + DOX), doxorubicin in a dose of 0.072 mg/rat, which is equivalent to the human dose 20 mg/m2 according to Barnes & Paget [19] once weekly for three weeks. Group (6) hepatocellular carcinoma model treated with both Nano-Se and doxorubicin in the same previous mentioned dose (HCC + Nano-Se + DOXO).

At the end of the period of treatment animals of all experimental groups were sacrificed by cervical dislocation and samples were collected. After that, the Samples were tested for DNA fragmentation, micronucleus test and chromosome aberrations and DNA damage in liver cells by comet assay.

2.5. Bone marrow chromosomal aberration assay

At first, the rats were injected with colchicine (4 mg/kg b.wt) two hours before sacrifice. Metaphase cells were prepared according to the standard technique of Preston et al. [20]. Bone marrow cells were aspirated from both femurs of each animal, and then the cells were centrifuged at 1000 rpm for 10 min. and resuspended in pre-warmed hypotonic solution (0.075 M potassium chloride) for 20 min at 37 °C. The samples were centrifuged and fixed in cold 3:1 methanol: glacial acetic acid. Each sample was washed five times with fixative then the slides were stained in 10% buffered Giemsa (pH 7.0), air-dried and mounted in DPX. Chromosome aberrations were identified according to criteria described by Savage [21].

2.6. The micronucleus test

Bone marrow slides were prepared according to the method described by Hayashi et al. [22]. The bone marrow was washed with 1 ml of fetal calf serum and then smeared on clean slides. The slides fixed in methanol for 5 min after drying followed by staining in May-Grunwald-Gemisa for 5 min, at least 2000 polychromatic erythrocytes (PCEs) per animal were examined for the presence of micronuclei.

2.7. DNA fragmentation assay

DNA fragmentation was measure by spectrophotometer using diphenylamine (DPA) method, according to the method of Perandones et al. [23] with some modifications. liver was homogenized in lyses buffer containing 5 mM Tris–HCl, pH 8.0, 20 mM EDTA and 0.5% Triton X-100. Centrifuged at 1500 × g for 20 min. Pellets were resuspended in 0.5 N perchloric acid and 5.5 N perchloric acid was added to supernatant, centrifuged again at 1500 × g for 10 min to remove proteins. Samples were heated at 90 ∘C and after cool reacted with diphenylamine (DPA) for 16–20 h at room temperature. Absorbance was measured at 600 nm using a UV-double beam spectrophotometer (Shimdazu 160 A). DNA fragmentation in samples = (frag. DNA in sup.)/[(frag. DNA in sup. + intact DNA in pellet)] were expressed as percentage of total DNA appearing in the supernatant fraction.

2.8. Detection of oxidative DNA damage (comet assay)

According to the method of Singh et al. [24], 0.5 g of crushed samples were transferred to 1 ml ice-cold PBS. This suspension was stirred for 5 min and filtered. 100 µl of cell suspension was mixed with 600 µl of low-melting agarose (0.8% in PBS). 100 µl of this mixture was spread on pre-coated slides which immersed in lyses buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15 min. The slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The electrophoresis conditions were 2 V/cm for 2 min and 100 mA. Staining with ethidium bromide 20 µg/ml. at 4 °C.

2.8.1. Comet capture and analysis

A total of 100 randomly captured comets from each slide were examined at 400 x magnification using a fluorescence microscope connected to CCD camera to an image analysis system [comet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK)]. A computerized image analysis system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components and then evaluates the range of derived parameters. To quantify the DNA damage tail length (TL), the percentage of migrated DNA (Tail DNA%) and tail moment (TM) were evaluated. Tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometers. It was calculated from the centre of the cell. Finally, the program calculates tail moment.

Table 1
Effect of Nano-Se on chromosomal aberrations in the different experimental groups.

Treatment	Structural aberrations							Numerical aberrations			Total aberrations
	Gap	Break	Deletion	Fragment	C.A	End.	Total structural	Aneuploidy	Polyploidy	Total numerical	
Control	0.50 ± 0.28^{c}	0.50 ± 0.28^{b}	0^{c}	0^{c}	0.50 ± 0.28^{cd}	0^{b}	1.50 ± 0.28^{d}	0.75 ± 0.25^{b}	0.50 ± 0.28^{cd}	1.25 ± 0.25^{cd}	2.75 ± 0.47^{d}
Nano-Se	0.75 ± 0.47^{bc}	0.50 ± 0.28^{b}	0^{c}	0^{c}	0.25 ± 0.25^{d}	0^{c}	1.50 ± 0.64^{d}	0.50 ± 0.29^{b}	0.25 ± 0.25^{d}	0.75 ± 0.25^{d}	2.25 ± 0.75^{d}
HCC	2.50 ± 0.28^{a}	1.75 ± 0.25^{a}	1.75 ± 0.25^{a}	1.75 ± 0.25^{a}	5.0 ± 0.40^{a}	1.25 ± 0.25^{a}	14.0 ± 0.70^{a}	4.25 ± 0.25^{a}	3.0 ± 0.40^{a}	7.25 ± 0.25^{a}	21.25 ± 0.94^{a}
HCC + Nano-Se	0.50 ± 0.28^{c}	0.50 ± 0.28^{b}	0.50 ± 0.28^{bc}	0^{c}	0.75 ± 0.25^{cd}	0^{c}	2.25 ± 0.25^{d}	0.75 ± 0.25^{b}	0.75 ± 0.25^{cd}	1.50 ± 0.28^{cd}	3.75 ± 0.25^{d}
HCC + DOX	1.75 ± 0.25^{ab}	1.50 ± 0.28^{a}	1.25 ± 0.25^{ab}	0.75 ± 0.25^{b}	1.75 ± 0.25^{b}	0.75 ± 0.25^{ab}	7.75 ± 0.25^{b}	1.25 ± 0.47^{b}	1.75 ± 0.25^{b}	3.0 ± 0.40^{b}	10.75 ± 0.62^{b}
HCC + Dox + Nano-Se	1.0 ± 0.40^{bc}	0.25 ± 0.25^{b}	1.0 ± 0.40^{ab}	0.50 ± 0.28^{bc}	1.25 ± 0.25^{bc}	0.25 ± 0.25^{bc}	4.25 ± 0.47^{c}	1.0 ± 0.40^{b}	1.25 ± 0.25^{bc}	2.25 ± 0.62^{bc}	6.50 ± 0.86^{c}

All data are represented as mean ± SE. Mean with different letters in each column were significantly different using analysis of variance test at $P \leq 0.05$.

2.9. Statistical analysis

Statistical analysis was carried out with SPSS software. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for comparison between different treatments. The values were expressed as mean ± S.E and differences were considered as significant when $P \leq 0.05$.

3. Results

3.1. Chromosome aberrations analysis

Statistical analysis of bone marrow indicated different types of chromosomal aberrations, which included structural aberrations such as (gap, break, deletions, fragments, centromeric attenuations and endomitosis) and numerical aberrations such as (prediploidy and polyploidy).

The results in (Table 1) showed that, the frequencies of structural and numerical chromosomal aberrations were significantly ($P \leq 0.05$) increased in hebatocellular carcinoma model group (HCC) comparing to both healthy control and nanoselenium groups. In contrast, the hebatocellular carcinoma model group treated with either nanoselenium (HCC + Nano-Se) or DOX (HCC + DOX) showed significant decrease ($P \leq 0.05$) in most types of chromosomal aberrations comparing with HCC group. However, by comparing the HCC group treated with nanoselenium and that treated with DOXO, it appeared that, nanoselenium caused more decrease in the frequencies of total structural and numerical aberrations. On the other hand, combination treatment of hebatocellular carcinoma animals with Doxorubicin and Nano-Selenium (HCC + DOX + Nano-Se) had low frequencies of total structural and numerical chromosome aberrations compared to HCC group.

3.2. Micronucleus analysis

The data in (Table 2) showed the percentage of micronucleated polychromatic erythrocytes (MNPCEs) formation in bone marrow cells of rats. The results indicated that, there was no significant reduction in MNPCEs in nanoselenium group comparing with vehicle control group. In contrast, MNPCEs frequencies were significantly increased in hebatocellular carcinoma rat model (HCC) comparing to healthy control and nanoselenium groups. Although there were significant difference in MNPCEs frequencies between Nano-Se, DOX and DOX + Nano-Se treated groups, however, Nano-Se showed the best reduction in the frequencies of MNPCEs. However, treated group with nanoselenium (HCC + Nano-Se) showed the highest decreased in the frequencies of MNPCEs.

3.3. DNA fragmentation

The results in (Table 3) showed the effect of nanoselenium and doxorubicin drugs on hepatocellular carcinoma animals model. In

Table 2
Effect of Nano-Se on the rate of micronucleated polychromatic erythrocytes (MNPCE) in the different experimental groups.

Treatment	MNPCE
Control	4.0 + 0.41^{e}
Nano-Se	3.25 + 0.25^{e}
HCC	34.0 + 0.40^{a}
HCC + Nano-Se	14.25 + 0.47^{d}
HCC + DOX	27.0 + 0.40^{b}
HCC + Dox + Nano-Se	22.50 + 1.04^{c}

All data are represented as mean ± SE. a,b,c,d,e. Mean with different letters in each column were significantly different using analysis of variance test at $P \leq 0.05$.

Table 3
Effect of Nano-Se on DNA fragmentation in the different experimental groups.

Treatment	Percentages of DNA fragmentation
Control	11.76 ± 0.13^{e}
Nano-Se	11.03 ± 0.24^{e}
HCC	44.37 ± 1.56^{a}
HCC + Nano-Se	17.63 ± 0.92^{d}
HCC + DOX	34.09 ± 1.73^{b}
HCC + DOX + Nano-Se	26.54 ± 1.24^{c}

All data are represented as mean ± SE. a,b,c,d,eMean with different letters in each column were significantly different using analysis of variance test at $P \leq 0.05$.

comparison with the negative control group, there was significant increase ($p \leq 0.05$) in rates of DNA fragmentation in HCC rat model. On the other hand, there were significant differences between all treated groups, but the treatment with nanoselenium was more effective in decreasing the rates of DNA fragmentation than other treatments.

3.4. DNA damage in liver cells by comet assay

The data in (Table 4 and Fig. 2) represented the DNA damage in liver cells of all experimental groups that evaluated with tail length, tail DNA% and tail moment.

The mean value of tail length in hepatocellular carcinoma rat model (Fig. 2B) was significantly increased compared to control and nanoselenium groups (2 A). On the other hand, the tail length percentages significantly decreased ($P \leq 0.05$) in all treated groups comparing to HCC model group.

As shown in Table 4, the extent of DNA damage that measured by tail DNA% and tail moment increased rapidly in hepatocellular carcinoma group when compared to healthy groups. Meanwhile, HCC animals treated with Nano-Se, doxorubicin alone or combined together showed a significant decreased ($P \leq 0.05$) in DNA damage but not reach to control percentage.

On the other hand, the treatment with nanoselenium either alone or in combination with DOX was more effective than treatment with doxorubicin alone.

4. Discussion

The most life-threatening human cancers in the world is HCC, which considered the major malignant tumor of the liver in adults and is the most common cause of death in people with cirrhosis [25]. There are several causes for HCC, e.g. alcohol addiction, which cause many medical complications and alcoholic liver disease (ALD) which is characterized by fatty liver, hepatitis, fibrosis and cirrhosis. If ALD remains untreated, the disease develops to HCC [26]. Also exposure to aflatoxin can develop HCC especially in Egypt, these by DNA damage in liver cells and mutation in p53 which is the tumor suppressor gene [27].

In this study, NDEA that used to HCC induction showed increase in frequencies of chromosomal aberrations and micronuclei formation. These results in agreement with Gupta et al. [28]. They suggested that this maybe due to increase the oxidative stress in liver [29]. The present results also in harmony with Buitrago-Molina et al. [3]who found that HCC frequently causes inflammation and chronic injury which promote the damage of DNA and chromosomal aberrations, Recurrent chromosomal aberrations are common in malignant solid tumors; many of these chromosome aberrations are potential diagnostic or prognostic markers [30,31]. Chromosomal aberrations may cause the initiation stages of carcinogenesis, so it is very importance to studying chromosome aberrations as a relevant biological endpoint to know the risks result from exposure to mutagenic carcinogens [32].

The current results showed increase in DNA damage in HCC and this may be because that HCC activate inflammatory cells, causing release of free radicals such as reactive oxygen species (ROS) and nitric oxide reactive species (NOS) which cause DNA damage and cancer [33]. ROS and other oxidative stress lead to death by continuation of chronic inflammatory responses and fibrogenesis [34]. In addition, ROS cause necrosis and apoptosis of liver cells [35,36].

In the present study, the treatment of HCC rat model with nanoselenium caused significant decrease in chromosomal aberrations, micronuclei formation and DNA damage compared with HCC group, which similar to results reported by Abd El-Rahim et al. [37] where the nanoselenium decreased the number of chromosomal aberrations and micronuclei formation. The current result is in keeping with that of Liu et al. [38] who suggested that selenium might delay NDEA-induced hepatocarcinogenesis in rats. Also, Novoselov et al. [39] and Popova [40] found that selenium inhibited hepatocarcinogenesis and decreased the proliferation of cell in mice that caused liver cancer.

On the other hand, HCC rat model treated with DOX had more frequencies in all parameters than that treated with Nano-Se. Although Dox has been regarded as one of the most effective chemotherapy drugs for cancer treatment [41] but it is the most common cytotoxic drug [42]. This may be due to that Dxorubicin has a metabolic activity that increase the generation of free radicals and induction of oxidative stress [43] and it causes an imbalance between ROS and antioxidant enzymes causing damage in liver cell [32]. Doxorubicin interacts with DNA by intercalation and inhibition of macromolecular biosynthesis [44–46]. This inhibits topoisomerase II enzyme which important in the transcription of DNA and broken the chain of DNA, so preventing the DNA double helix from being resealed and thereby stopping the process of replication [44].

In view of the present data, the treatment of HCC rat model with doxorubicin and Nano-Se resulted in a significant decrease in chromosomal aberrations, micronuclei formation and DNA damage comparing to treatment with Dox only. Our results was in harmony with Rastgoo and Sadeghi [47] who found that the combination of doxorubicin with nanoselenium better than doxorubicin alone. The cause of this may be due to the high levels of plasma corticosterone [48] as animals subjected to oxidative stress in treatment with doxorubicin alone. So, Nano-Se protect against toxicities of anticancer drug in vitro and in vivo [49]. Selenium has been effective in reducing the cancer in animal models as well as human clinical trials [50].

Table 4
Effect of Nano-Se on the Parameters of DNA damage in the comet assay in the different experimental groups.

Treatment	% tailed	Untailed%	Tail length(μm)	Tail DNA %	Tail moment
Control	6.25 ± 0.47^{d}	93.75 ± 0.47^{a}	1.69 ± 1.88^{d}	1.98 ± 0.27^{c}	3.39 ± 0.69^{d}
Nano-Se	5.75 ± 0.47^{d}	94.25 ± 0.47^{a}	1.67 ± 0.19^{d}	1.87 ± 0.34^{c}	3.18 ± 0.72^{d}
HCC	23.0 ± 0.70^{a}	77.0 ± 0.70^{d}	6.13 ± 0.24^{a}	5.25 ± 0.08^{a}	32.22 ± 1.38^{a}
HCC + Nano-Se	13.50 ± 0.64^{c}	86.50 ± 0.64^{b}	2.96 ± 0.32^{c}	3.11 ± 0.24^{b}	9.0 ± 0.44^{c}
HCC + DOX	17.0 ± 0.40^{b}	83.0 ± 0.40^{c}	4.27 ± 0.36^{b}	4.55 ± 0.20^{a}	19.61 ± 2.29^{b}
HCC + DOX + Nano-Se	15.75 ± 0.85^{b}	84.25 ± 0.85^{c}	3.45 ± 0.49^{bc}	3.50 ± 0.32^{b}	12.16 ± 2.12^{c}

All data are represented as mean ± SE. a,b,c,d,eMean with different letters in each column were significantly different using analysis of variance test at $P \leq 0.05$.

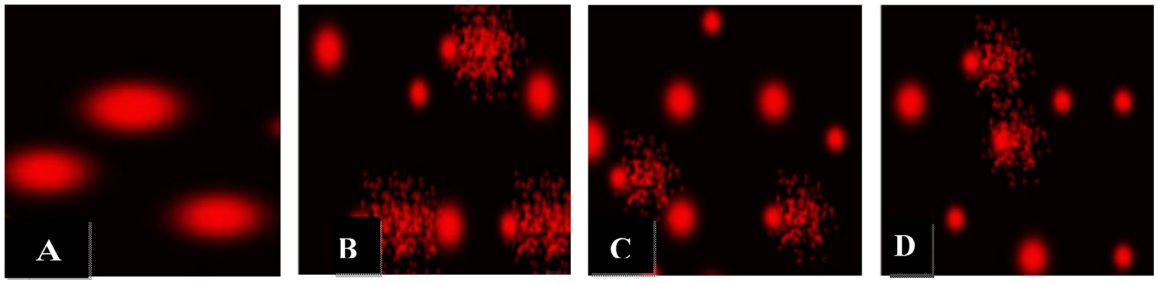

Fig. 2. Comet assay prepared of rat liver showing varying extent of DNA damage in different treatments.
A: Intact cells; B: tail length > 2X the diameter of nucleus; C: tail length between 1X and 2X the diameter of nucleus and D: tail length < diameter of nucleus.

5. Conclusion

The present study clarified the potential role of selenium nano-particles and Doxorubicin in repression of genotoxicity in hepatocellular carcinoma rat model. They cause decreased in chromosomal aberrations, micronuclei formation as well as DNA damage percentage, but, selenium nano-particles was more effective than Doxorubicin. However, more scientific studies are needed to support the use of Nano-Se for human disease prevention or lifespan extension.

Conflict of interest

None.

References

[1] F.X. Bosch, J. Ribes, M. Diaz, R. Cleries, Primary liver cancer: worldwide incidence and trends, Gastroenterology 127 (2004) 5–16.
[2] L.B. Seeff, Natural history of chronic hepatitis C, Hepatology 36 (2002) 35–46.
[3] L.E. Buitrago-Molina, S. Marhenke, T. Longerich, et al., The degree of liver injury determines the role of p21 in liver regeneration and hepatocarcinogenesis, Hepatology 58 (2013) 1143–1152.
[4] R.C. Ong, J. Lei, R.K. Lee, et al., Polyphyllin D induces mitochondrial fragmentation and acts directly on the mitochondria to induce apoptosis in drug-resistant HepG2 cells, Cancer Lett. 261 (2008) 158–164.
[5] S. Nie, Y. Xing, G.J. Kim, J.W. Simons, Nanotechnology applications in cancer, Annu. Rev. Biomed. Eng. 9 (2007) 257–288.
[6] M.K. Teli, S. Mutalik, G.K. Rajanikant, Nanotechnology and nanomedicine: going small means aiming big, Curr. Pharm. Des. 16 (2010) 1882–1892.
[7] J.S. Zheng, S.Y. Zheng, Y.B. Zhang, et al., Sialic acid surface decoration enhances cellular uptake and apoptosis-inducing activity of selenium nanoparticles, Colloids Surf. B Biointerfaces 83 (2011) 183–187.
[8] S.K. Sahoo, S. Parveen, J.J. Panda, The present and future of nanotechnology in human health care, Nanomedicine 3 (2007) 20–31.
[9] L. Giannitrapani, M. Soresi, M. Bondì, G. Montalto, M. Cervello, Nanotechnology applications for the therapy of liver fibrosis, World J. Gastroenterol. 20 (2014) 7242–7251.
[10] R. Sinha, K. El-Bayoumy, Apoptosis is a critical cellular event in cancer chemoprevention and chemotherapy by selenium compounds, Curr. Cancer Drug Targets 4 (2004) 13–28.
[11] A. Nel, T. Xia, L. Madler, N. Li, Toxic potential of materials at the nanolevel, Science 311 (2006) 622–627.
[12] H. Wang, J. Zhang, H. Yu, Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice, Free Radic. Biol. Med. 42 (2007) 1524–1533.
[13] J. Zhang, X. Wang, T. Xu, Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with semethylselenocysteine in mice, Toxicol. Sci. 101 (2008) 22–31.
[14] H.H. Ahmed, W.B. Khalil, A.H. Hamza, Molecular mechanisms of Nano-selenium in mitigating hepatocellular carcinoma induced by N-nitrosodiethylamine (NDEA) in rats, Toxicol. Mech. Methods 24 (2014) 593–602.
[15] R. Brigelius-Flohe, M. Maiorino, Glutathione peroxidases, Biochim. Biophys. Acta-Gen. Subj. 1830 (2013) 3289–3303.
[16] P. Horky, B. Ruttkay-Nedecky, L. Nejdl, L. Richtera, N. Cernei, M. Pohanka, P. Kopel, J. Skladanka, P. Hloucalova, P. Slama, P. Nevrkla, V. Mlejnkova, V. Klusonova, R. Kizek, V. Adam, Electrochemical methods for study of influence of selenium nanoparticles on antioxidant status of rats, Int. J. Electrochem. Sci. 11 (2016) 2799–2824.
[17] C. Dwivedi, C.P. Shah, K. Singh, M. Kumar, P.N. Bajaj, An organic acid-induced synthesis and characterization of selenium nanoparticles, J. Nanotechnol. 2011 (2011) 1–6.
[18] K. Karimov, F.K. Inoyatova, M.T. Mukhamedova, Changes in some indices of the synthesis of nitric oxide during the early stages of hepatocarcinogenesis, Exp. Toxicol. Pathol. 55 (2003) 17–19.
[19] J.M. Barnes, G.E. Paget, Mechanisms of toxic action, Prog. Med. Chem. 4 (1965) 18–38.
[20] R.J. Preston, B.D. Dean, S. Galloway, H. Holden, A.F. McFee, M. Shelly, Mammalian in vivo cytogenetic assays: analysis of chromosome aberrations in bone marrow cells, Mutat. Res. 189 (1987) 157–165.
[21] J.R.K. Savage, Classification and relationship of induced chromosomal structural changes, J. Med. Genet. 12 (1975) 103–122.
[22] M. Hayashi, T. Sofuni, M. Ishidate Jr, An application of acridine orange fluorescent staining to the micronucleus test, Mutat. Res. 120 (1983) 241–247.
[23] C.E. Perandones, V.A. Illera, D. Peckham, L.L. Stunz, R.F. Ashman, Regulation of apoptosis in vitro in mature murine spleen T cells, J. Immunol. 151 (1993) 3521–3529.
[24] N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175 (1988) 184–191.
[25] A. Forner, J.M. Llovet, J. Bruix, Hepatocellular carcinoma, The Lancet 379 (2012) 1245–1255.
[26] V. Purohit, D. Russo, Cellular and molecular mechanisms of alcoholic hepatitis, Alcohol 27 (2002) 3–6.
[27] A. El-Zayadi, H. Abaza, S. Shawky, et al., Prevalence and epidemiological features of hepatocellular carcinoma in Egypt – a single center experience, Hepatol. Res. 19 (2001) 170–179.
[28] P. Gupta, M. Bansal, A. Koul, Lycopene modulates initiation of N-nitrosodiethylamine induced hepatocarcinogenesis: studies on chromosomal abnormalities, membrane fluidity and antioxidant defense system, Chem. Biol. Interact. 206 (2013) 364–374.
[29] H.O. El Mesallamy, N.S. Metwally, M.S. Soliman, et al., The chemopreventive effect of Ginkgo biloba and Silybum marianum extracts on hepatocarcinogenesis in rats, Cancer Cell Int. 11 (2011) 38–50.
[30] C.P. Karakousis, P. Dal Cin, C. Turc-Carel, J. Limon, A.A. Sandberg, Chromosomal changes in soft-tissue sarcomas. A new diagnostic parameter, Arch Surg. 122 (1987) 1257–1260.
[31] J.A. Fletcher, H.P. Kozakewich, F.A. Hoffer, J.M. Lage, N. Weidner, R. Tepper, G.S. Pinkus, et al., Diagnostic relevance of clonal cytogenetic aberrations in malignant soft-tissue tumors, N. Engl. J. Med. 324 (9) (1991) 436–442.
[32] E.E. Essick, F. Sam, Oxidative stress and autophagy in cardiac disease, neurological disorders, aging and cancer, Oxid. Med. Cell. Longev. 3 (2010) 168–177.
[33] T. Chen, Y.S. Wong, Selenocystine induces apoptosis of A375 human melanoma cells by activating ROS-mediated mitochondrial pathway and p53 phosphorylation, Cell. Mol. Life Sci. 65 (2008) 2763–2775.
[34] E. Novo, M. Parola, Redox mechanisms in hepatic chronic woundhealing andfibrogenesis, Fibrogen. Tissue Repair 1 (2008) 5.
[35] J. Wu, M.A. Zern, Hepatic stellate cells: a target for the treatment of liver fibrosis, J. Gastroenterol. 35 (2000) 665–672.
[36] R. Bataller, R.F. Schwabe, Y.H. Choi, et al., NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis, J. Clin. Invest. 112 (2003) 1383–1394.
[37] A.H. Abd El-Rahim, O.M. Abd-Elmoneim, N.A. Hafiz, Assessment of Antigenotoxic Effect of Nanoselenium and Metformin on Diabetic Rats, Jordan J. Biol. Sci. 10 (2017) 159–165.
[38] J.G. Liu, H.J. Zhao, Y.J. Liu, Y.W. Liu, X.L. Wang, Effect of two selenium sources on hepatocarcinogenesis and several angiogenic cytokines in diethylnitrosamine-induced hepatocarcinoma rats, J. Trace Elem. Med. Biol. 26 (2012) 255–261.
[39] S.V. Novoselov, D.F. Calvisi, V.M. Labunskyy, V.M. Factor, B.A. Carlson, D.E. Fomenko, M.E. Moustafa, D.L. Hatfield, V.N. Gladyshev, Selenoprotein deficiency and high levels of selenium compounds can effectively inhibit hepatocarcinogenesis in transgenic mice, Oncogene 24 (2005) 8003–8011.
[40] N.V. Popova, Perinatal selenium exposure decreases spontaneous liver tumorigenesis in CBA mice, Cancer Lett. 179 (2002) 39–42.
[41] R.B. Weiss, The anthracyclines: will we ever find a better doxorubicin? Semin. Oncol. 19 (1992) 670–686.
[42] H. Nakamura, T. Hashimoto, H. Oi, S. Sawada, Transcatheter oily chemoembolization of hepatocellular carcinoma, Radiology 170 (1989) 783–786.
[43] K.H. Kim, G.Y. Oudit, P.H. Backx, Erythropoietin protects against doxorubicin-induced cardiomyopathy via a phosphatidylinositol 3-kinase-dependent pathway, J. Pharmacol. Exp. Ther. 324 (2008) 160–169.
[44] O. Tacar, P. Sriamornsak, C.R. Dass, Doxorubicin: an update on anticancer

molecular action, toxicity and novel drug delivery systems, J. Pharm. Pharmacol. 65 (2013) 157–170.
[45] F.A. Fornari, J.K. Randolph, J.C. Yalowich, M.K. Ritke, D.A. Gewirtz, Interference by doxorubicin with DNA unwinding in MCF-7 breast tumor cells, Mol. Pharmacol. 45 (1994) 649–656.
[46] R.L. Momparler, M. Karon, S.E. Siegel, F. Avila, Effect of adriamycin on DNA, RNA, and protein synthesis in cell-free systems and intact cells, Cancer Res. 36 (1976) 2891–2895.
[47] S. Rastgoo, A.A. Sadeghi, Effect of nano-selenium on plasma antioxidant status and reproductive system function of female rats exposed to oxidative stress induced by doxorubicin, Biol. Forum –Int. J. 7 (2015) 187–191.
[48] M.P. Artykova, I.F. Perfileva, E.Sh. Chumburidze, Excretion of adrenaline, noradrenaline and luteinizing hormone during treatment with arsenate mineral water of chronic adnexitis, VoprKurortolFizioter Lech FizKult 3 (1977) 72–74.
[49] F. Gao, Q. Yuan, L. Gao, P. Cai, H. Zhu, R. Liu, Y. Wang, Y. Wei, G. Huang, J. Liang, X. Gao, Cytotoxicity and therapeutic effect of irinotecan combined with selenium nanoparticles, Biomaterials 35 (2014) 8854–8866.
[50] K. El-Bayoumy, R. Sinha, Molecular chemoprevention by selenium: a genomic approach, Mutat. Res. 591 (2005) 224–236.