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BIOLOGICALLY PRODUCED NANO-SELINUM REDUCES INITIATION STAGE OF DIETHYL NITROSAMINE HEPATOCARCINOGENESIS IN RATS

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ABSTRACT

Biologically produced Nano-selenium (Nano-se) considers one of the less toxic element in comparison with the other forms of selenium. The present study evaluated the protective effect of biologically Nano-se against early hepatocarcinogenesis induced by diethyl nitrosamine (DEN) for 1 week in rats. Forty male Wister rats were divided into four groups as follows: Group 1 treated with intraperitoneal (i.p.) injection with physiological saline once and kept as control; group 2 treated with DEN alone (200 mg/kg) i.p. injection once; group 3 treated with both DEN and Nano-Se and group 4 treated with Nano-Se alone (0.5 mg/kg) orally by gastric intubation. DEN administration did not alter the erythrogram but induced significant changes in leukogram represented by increase in total leukocytic count (TLC), neutrophil and monocyte cell counts together with significant reduction in lymphocyte and eosinophil cells count ($P \leq 0.05$). DEN induced hepatotoxicity noticed through an elevation of serum hepatic enzymes such as AST, ALT and γ-GT. Additionally, an increase in the oxidative stress marker as malondialdehyde content (MDA) and reduction of antioxidant parameters such as catalase (CAT) and reduced glutathione (GSH) were observed. Moreover, DEN was associated with the formation of putative hepatic foci indicated with over expression of glutathione transeferase –placental form (GST-P) immunostaining. Interestingly, Nano-Se clearly alleviated the negative impacts of DEN induced hepatotoxicity via returning leukogram to normal condition, suppressed the elevated of serum hepatic enzymes, oxidative stress markers, decrease positive GST-P foci and remarkably increased the antioxidant capacity. Therefore, biological produced Nano-se might be considered as an effective strategy in hepatic cancer chemoprevention.

Key words: *Nano-Se*, initiation stage, oxidative stress, antioxidant properties.

INTRODUCTION

Hepatocellular carcinoma (HCC) represents one of the most lethal worldwide malignancies in human. Environmental disturbance and pollution among the mean causes of HCC.

In Egypt, the incidence of HCC has been increasing especially in the last ten years, and usually attributed to the increased prevalence of numerous risk factors such as the higher prevalence of hepatitis B and C viral infection, aflatoxins, heavy metals and organochlorine pesticides and their combinations in contaminated food and water (Anwar *et al.,* 2008; Abdo *et al.,* 2013; Shaker *et al.,* 2013). Chemical carcinogens with hepatitis viruses B and C are involved in a multistage process of hepatocellular carcinogenesis involving genetic and epigenetic pathways (Gu 2012). There are two primary driving forces behind the development and exacerbation of HCC include oxidative stress and unresolved inflammation (Bishayee 2012).

DEN is a potent hepatocarcinogen, has been detected in various foodstuffs such as milk products, meat products, soft drinks, and alcoholic beverages (Levallois *et al.,* 2000). Since DEN itself does not exert carcinogenicity, it needs to be activated by cytochrome P450 (CYP) enzymes in the liver, which resulted in DNA-adducts formation via DNA alkylation mechanism. Although several CYP enzymes are proposed to catalyze DEN (Verna *et al.,* 1996). CYP2E1 plays an essential role in the metabolism of DEN. It is noteworthy that CYP2E1-deficient mice reveal lower tumor incidence and multiplicity in DEN hepatocarcinogenesis in comparison with wild type mice (Williams 2001; Kang *et al.,* 2007). Chemoprevention could be one of the strategies to decrease the risk of HCC

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development especially in carriers of hepatitis B and C viruses (Stagos *et al.,* 2012).

Selenium is an essential micronutrient that is important for the antioxidant effects of the selenoproteins. At least two different enzyme families of selenoproteins are present: glutathione peroxidases and thioredoxin reductases. Selenium takes part in free radicals scavenging and being a cofactor of these antioxidant enzymes, thus, protecting cells, membranes and cell organelles, enzymes and nucleic acids from the harmful effects of reactive oxygen species (ROS) (Jia *et al.,* 2015). The concentration of reactive oxygen species in various tissues and cells such as neutrophil granulocytes and monocytes is controlled by Selenoproteins (Szuchman-Sapir *et al.,* 2012). Which have an essential physiological role in immune responses by producing huge amounts of ROS against microbes. The antioxidant defense capacity of immune cells could be exhausted by either elevated oxidative stress or depleted protection by selenoproteins due to selenium deficiency causing immunotoxicity (Gust *et al.,* 2013). Selenium is an important micronutrient at low concentration, but being toxic by increased level(Wang *et al.,* 2007). It also acts as a chemo preventive and chemotherapeutic agent for human cancer (Feng *et al.,* 2014).

Recently, Nano-materials have been received much more attention. They exhibit unique superiority of its physical properties such as great specific surface area, high surface activity, a lot of catalytic efficiency and potent absorptive ability due to the interaction between $–NH_2$, C=O, −COO, and –C–N– groups of proteins and the nanoparticles of Se (Hassanin *et al.,* 2013). The unique absorptive ability which increases the bioavailability and lower toxicity compared with selenite in chickens (Wang *et al.,* 2009), rats (Jia *et al.,* 2005), Sheep (Shi *et al.,* 2011a), mice (Wang *et al.,* 2007) and goats (Shi *et al.,* 2011b). Subsequent studies described that a novel elemental Se source called nano-Se possessed a higher efficiency than selenite, seleno-methionine, and methyl-selenocysteine in up regulating selenoenzymes in mice and rats (Wang *et al.,* 2007). Nano-Se is a bright red, highly stable and of Nano meter size in the redox state of zero (Se0) (Zhang *et al.,* 2001). Several mechanisms have been postulated to elucidate the anticancer activity of selenium, including induction of cell apoptosis, inhibition of cell proliferation, modulation of redox state, detoxification of carcinogen, stimulation of the immune system and inhibition of angiogenesis (Zhao *et al.,* 2006). Biologically produced Nano-Se could be safer and more efficient than the ones produced by the chemical methods. Saleh (2014) stated that there are no adverse effects of using biologically produced Nano-Se in poultry nutrition. Selenium nanoparticles were found to be effective to inhibit the proliferation of human breast cancer cell line MCF-7 in a dose dependent manner (Ramamurthy *et al.,* 2013). Although antioxidative and ant-inflammatory properties of nano-selenium might be the basic mechanism of the anti-cancer effect. Therefore, the goal of the present experiment was to assess whether the biologically produced Nano-Se may reduce preneoplastic hepatic foci in rats exposed to DEN.For this purpose, hematological, serum biochemical and lipid peroxidation and antioxidant biomarkers, histopatholgical examination as well as assessment of glutathione *S*-transferase placental form-positive (GST-P) initiated cells were evaluated in DEN intoxicated rat.

MATERIALS AND METHODS

1. Chemicals:

DEN CAS No. 55-18-5, (purity > 99.0%) was purchased from Sigma-Aldrich Chemie GmbH (Hamburg, Germany). Selenium nano-particles (100-500 nm range - ready for use) was kindly obtained from Professor Mohsen Zommara, Department of Dairy Science, Faculty of Agriculture, Kafrelsheikh University, Egypt and Prof. JozsefProkisch, Center for Agricultural Sciences Institute of Bio- and Environmental Energy, University of Debrecen, Hungary. Se nanoparticles of 100 to 500 nm were prepared according to (Zommara 2007; Prokisch *et al.,* 2008). Briefly, selenium nanoparticles were prepared from pure yoghurt cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L.bulgaricus*) (NCAIM B 02206), *Streptococcus thermophilus* (*S. thermophilus*) (CNCM I-1670) obtained from the National Collection of Agricultural and Industrial Microorganisms, Budapest, Hungary (El-Baz and Zommara 2007). MRS media were supplemented with 20 ppm of filter-sterilized (SARTORIUS AG, Germany) selenium Se (IV) (sodium selenite, Na_2SeO_3. $5H_2O$, SIGMA-ALDRICH, Switzerland) and incubated at 40°C up to 24 h. The media centrifuged for 20 min at 4500 g at room temperature to spin down the bacteria cells. The cell pellets washed twice by Tris-HCl buffer (50mM, pH 7.5) and finally with ultra-pure water to get the Nano-Se particles fortified cell fraction. The Se content analyzed by inductively coupled plasma mass spectrometer (ICP-MS) (X series, THERMO Fisher Scientific, Germany).

2. Animals:

Forty male Wistar rats (6 weeks age-150- 160g weight) were obtained from the Animal House Institute of the National Research Center, Dokki, and Cairo, Egypt. The animals fed a standard diet (Al Wadi Co., Giza, Egypt), and accessed water and food *ad libitum*. The animals were housed in a plastic cage in an air-conditioned room at 25°C and with a 12-h

light/dark cycle. The animals used for the experiments after one-week acclimatization period. All animals handled measures were done in accordance with the regulation for care and use of experimental animals guidelines for the National Institutes of Health (NIH) and the experimental protocol approved by Animal Ethics Committee of Kafrelsheikh University – Egypt.

3. Experimental protocol

The experimental protocol was summarized in table 1, Forty adult male Wister rat were divided into 4 groups (n=10). Control group received a single I/P injection of saline at the 1st day of experiment. DEN group received a single (I/P) of DEN (200 mg/kg body weight) according to (Basak *et al.,* 2000) at the 1st day of experiment. DEN and Nano-Se group which administrated Nano-selenium (0.5 mg/kg body weight) dispersed in 0.5 mL PBS orally by stomach tube was given one hour before DEN administration from the beginning of the experiment for one week (Peng *et al.,* 2007) and received a single (I/P) of DEN (200 mg/kg body weight) at the 1st day of experiment. Nano-Se group received a daily oral administration of Nano-Se at dose of 0.5 mg/kg body weight of dispersed in 0.5 mL PBS orally by stomach tube.

Table 1: Summary of different rat groups and their treatment.

Group	Treatment	DEN	Nano-Se
1	**Control**	**-**	**-**
2	**DEN**	**+**	**-**
3	**DEN + Nano-Se**	**+**	**+**
4	**Nano-Se**	**-**	**+**

Diethylnitrosamine (200 mg/kg bw) ip once for 1 week (DEN). Nano-selenium (0.5 mg/kg bw) given orally by stomach tube daily for 1 week. (Nano-Se), 1 h before DEN dose (DEN + Nano-Se)

4. Body and liver weight:

Total body weight was measured on first and last day of the experiment. While, the liver was excised and weighed on the sacrifice.

5. Blood sample:

At the end of experimental period 7days (24 h after the last treatment of Nano-Se), blood samples were collected via retro-orbital Venus plexus under light ether anesthesia and immediately divided into two aliquots. The 1st one containing EDTA as anticoagulant for hematologicalanalysis and for estimation of reduced glutathione (GSH). The 2nd part without anticoagulant left to clot then centrifuged at 3000 rpm for 15 minutes. Sera were then, separated into aliquots for individual biochemical and antioxidant biomarkers (MDA and CAT) estimations and stored at -20°C. After blood collection, the rats were sacrificed by decapitation.

6. Hematological analysis:

The aliquot contained EDTA (1 mg/ml) used for assessing red blood corpuscles (RBCs), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Total leucocytic count (TLC) and differential leucocyte count (Weiss and Wardrop 2011).

7. Serumbiochemical assay:

The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) evaluated according to (Reitman and Frankel 1957) and gamma glutamyltransferase (γGT) was evaluated according to (Szasz 1969) were measured spectrophotometrically according to manufacturers' instructions.

8. Estimation of antioxidants and lipoperoxidation markers:

Measurement of MDA content as an indicator for lipid peroxidation was performed according to (Mihara and Uchiyama 1978). Antioxidant biomarkers as catalase (CAT) according to (Aebi 1984) and reduced glutathione (GSH) according to (Beutler *et al.,* 1963) were measured spectrophotometrically according to manufacturers' instructions.

9. Histopathology and immunohistochemistry:

At the end of the experimental period (7 days), rats were scrifiased by cervical dislocation, immediately liver sections from the right and median lobes fixed in 10% neutral-buffered formalin. The specimens then dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin wax. Next, the paraffin-embedded specimens were sectioned into4-µm samples for hematoxylin and eosin staining

(Sigma-Aldrich Chemie GmbH) and immune staining. Immunohistochemically staining for placental glutathione S-transferase using a polyclonal rabbit anti-rat GST-P antibody (cat. no. 311, Medical & Biological Laboratories Co., Ltd., Nagoya, Japan; 1:500 dilution) as previously described by (Abdo *et al.,* 2013). The number of positive cells were expressed as the percent of positive cells in about a total 1000 hepatocytes.

10. Statistical analysis

The data analyzed by one-way analysis of variance (ANOVA) with the General Linear Model using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). The significant differences among means of treatments compared by Tukey's multiple test. $P<0.05$ was set as limit of significance.

RESULTS

1. Body and liver weights:

In all the experimental groups, the rats survived throughout the experimental period with no observed severe adverse clinical effects. The final body and liver weights of the treated groups presented in Table (2). Notably, supplementation of Nano –Se decrease the absolute and relative liver weights that induced by the DEN. Meanwhile, the liver weights in Nano-Se treated group were within the normal limits.

Table 2: Body and liver weights after the 1 weeks of treatment.

Group	Treatment	No of rats	Body weight (g)	Liver weight	
				Absolute (g)	Relative (%)
1	Control	10	163.5±0.57 ab	6.26±0.57 ab	3.83±0.2 ab
2	DEN	10	164.0±1.70 a	7.33±0.78 a	4.47±0.24 a
3	DEN + Nano-Se	10	169.1±1.59 ab	6.24±1.00 ab	3.69±0.44 a
4	Nano-Se	10	168.0±1.04 a	7.06±1.02 a	4.2±0.44 a

Data are expressed as mean ± standard error (SEM), Different letters with each column indicate significant differences when compared to the control group at $p<0.05$ when compared with group.

2. Effect on Hematological Parameters

The effect of DEN intoxication as well as the protective effects of Nano-Se on hematological parameters are shown in Table 3.There was no significant difference on erythrogram in all the examined groups, while the leukogram, revealed significant increase in lymphocyte count (117.2%) in Nano-Se group in compared with control group ($P<0.05$). Administration of DEN significantly increased the total leucocytic count (TLC), neutrophils and monocyte counts (159.8,245.5 and 361.5% respectively) ($P<0.05$). Contrary to this, significant decline in lymphocytes and eosinophils counts were recorded (71.1 and 47.4% respectively) compared with the control group ($P<0.05$). Nano-Se co-supplementation significantly ameliorated the stress picture of leukogram and restored the elevated TLC,neutrophil and monocyte counts toward the normal value (66.9,43.8 and 49.2% respectively), improving the declined lymphocyte and eosinophil counts (138.5 and 198% respectively) compared to DEN treated group ($P\leq 0.05$).

Table 3: The effect of Nano-Se against DEN on hematological parameters.

Group	RBCs (x103/µl)	PCV (%)	Hb (gm/dl)	MCV (n)	MCH (pg)	MCHC (%)
Control	6.05±0.04	34.17±0.65	14.42±0.26	56.52±1.3	23.85±0.54	42.27±1.1
DEN	5.39±0.28	31.5±0.8	12.57±0.36	59.01±0.2	23.51±0.71	39.94±1.03
DEN + Nano-Se	5.91±0.13	32.71±0.71	13.03±0.35	55.37±0.49	22.06±0.54	39.93±1.29
Nano-Se	6.17±0.12	33.83±0.65	13.87±0.28	54.9±0.72	22.38±0.5	40.78±0.83

Group	TLC (x103/µl)	Lymphocytes (x103/µl)	Neutrophils (x103/µl)	Monocytes (x103/µl)	Eosinophils (x103/µl)
Control	9.52±0.22b	3.9±0.13b	4.71±0.0.10b	0.589±0.04c	0.321±0.042a
DEN	15.21±0.32a	2.35±0.38c	10.58±0.38a	2.129±0.33a	0.152±0.001b
DEN + Nano-Se	10.19±0.66b	3.64±0.32b	5.20±0.31b	1.047±0.22b	0.3010.033a
Nano-Se	10.07±0.72b	5.52±0.33a	3.50±0.38b	0.781±0.08c	0.266±0.042a

Different letters with each column indicate significant differences when compared to the control group at p<0.05. DEN, diethylintrosamine, Nano-Se, nano-selenium.

3. Serum biochemical analysis:

The impact of Nano-Se supplementation against DEN induced toxicity on serum enzymes is shown in Figure 1. There was no significant difference in serum levels of liver function marker enzymes in rats received only Nano-Se compared to the control group. However, DEN treated group showed significant increase of serum ALT, AST, and γ-GT enzymes (303.2, 300.9 and194.7 % respectively) compared to the control group (P<0.05). Moreover, Nano-Se co- administration significantly ameliorated the changes in the measured serum parameters (about 46.4, 61.02 and 58.6 5 % respectively) compared to the DEN-treated group (P<0.05). These results indicated that Nano-Se could effectively abolished DEN-induced hepatictoxicity.

4. Oxidative stress marker and antioxidant activities:

The influences of nano-Se supplementation against DEN on serum MDA content as well as antioxidant activity are presented in Figure.2. Rat group supplemented by Nano-Se only did not affect MDA values, however, it revealed a significantly enhanced GSH and CAT activities (about 134.8 and 110.8% respectively) compared to control group (P<0.05). DEN administration significantly (P<0.05) increased serum content of MDA (about 213.9%), while significantly (P<0.05) depleted GSH and CAT activities (about 45.3 and 63.3% respectively) compared to the control group. Nano-Se supplemented group with DEN restored the altered serum MDA (about 62.4%) and enhanced GSH and CAT activities (about 197.9 and 153.9% respectively) compared to the DEN group.

5. Histopathological and immunohistochemical findings

Histopathological and immunohistochemical of DEN and Nano-Se treated groups are illustrated in Figure. 3 and 4. Liver of control animals showed normal hepatocytes which arranged cords around the central vein. DEN-treated rats revealed congestion of central vein and blood sinusoids, hypertrophy of centro-lobular hepatocytes, vacuolation of their cytoplasm as well as periductal oval cell proliferation and an increase in mitotic figures (6.45±1.26 cell/mm2). Many of preneoplastic foci were also observed such as clear and eosinophilic types. Nano-Se was greatly decreased histopathological alterations induced by DEN administration such as decrease putative neoplastic foci, oval cell and significant decrease of the number of mitotic cells (2.61±0.85 cell/mm2) (P< 0.001). GST-P immunostaining was markedly expressed in DEN treated group within the neoplastic hepatocytes and also within the periductal oval cells, while animals treated with nano-Se revealed a significant decrease the neoplastic-GST-P positive cell (P< 0.001).

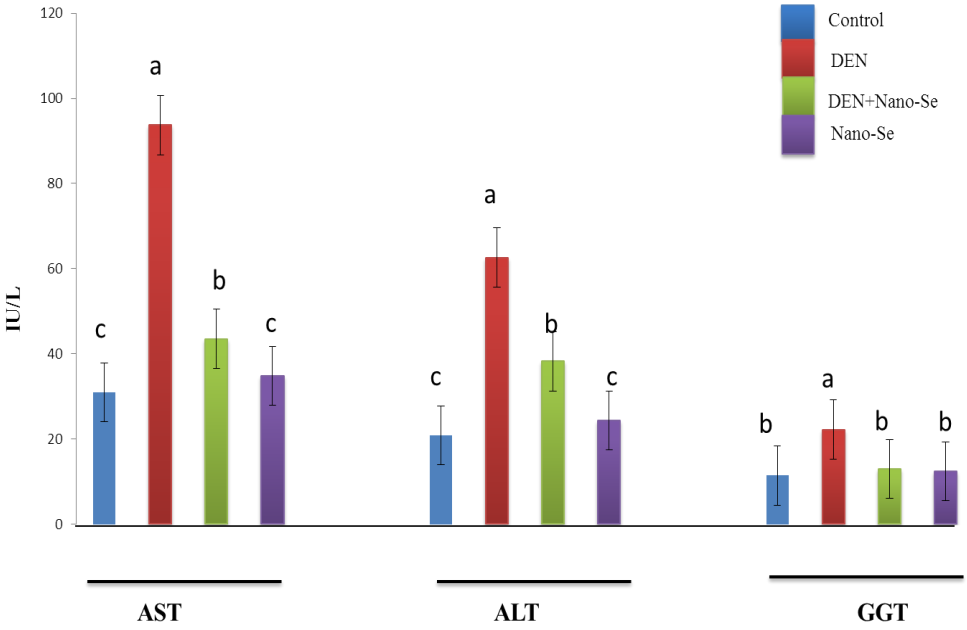


Figure.1 Serum AST,ALT,GGT activities in the control and the different treated groups. Data are presented as mean±SE. Statistical significance of the data was analyzed using SPSS programme (Statical package for Social Science) version 17. For comparison, one-way analysis of variance (ANOVA) test and post-comparison was carried out with Tukey's Range Test for post hoc analysis. Statistical significance Was acceptable at a level of ($P \leq 0.05$)

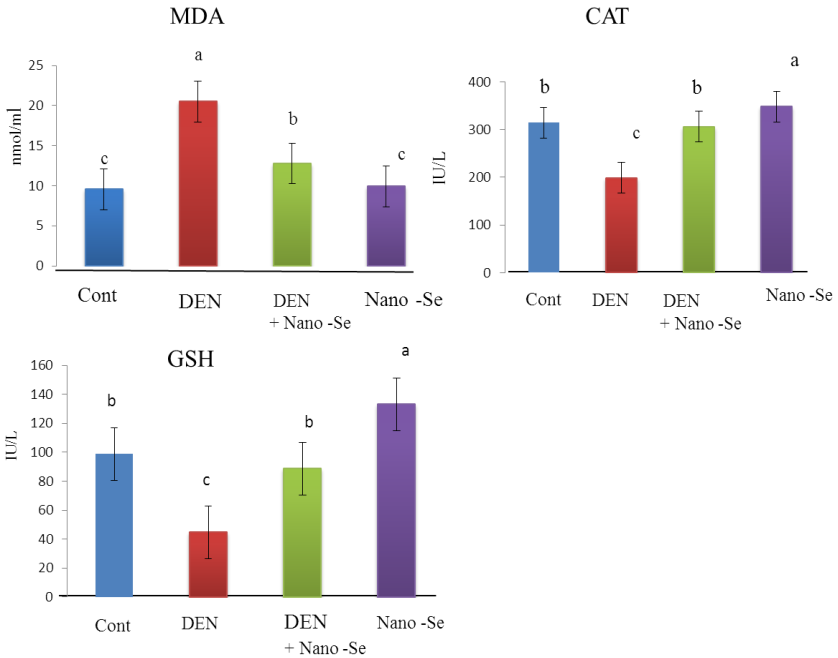


Figure .2. Serum MDA content as well as reduced glutathione (GSH) and catalase (CAT) in the control and the different treated groups. Data are presented as mean±SE. Statistical significance of the data was analyzed using SPSS programme (Statical package for Social Science) version 17. For comparison, one-way analysis of variance (ANOVA) test and post-comparison was carried out with Tukey's Range Test for post hoc analysis. Statistical significance Was acceptable at a level of ($P \leq 0.05$)

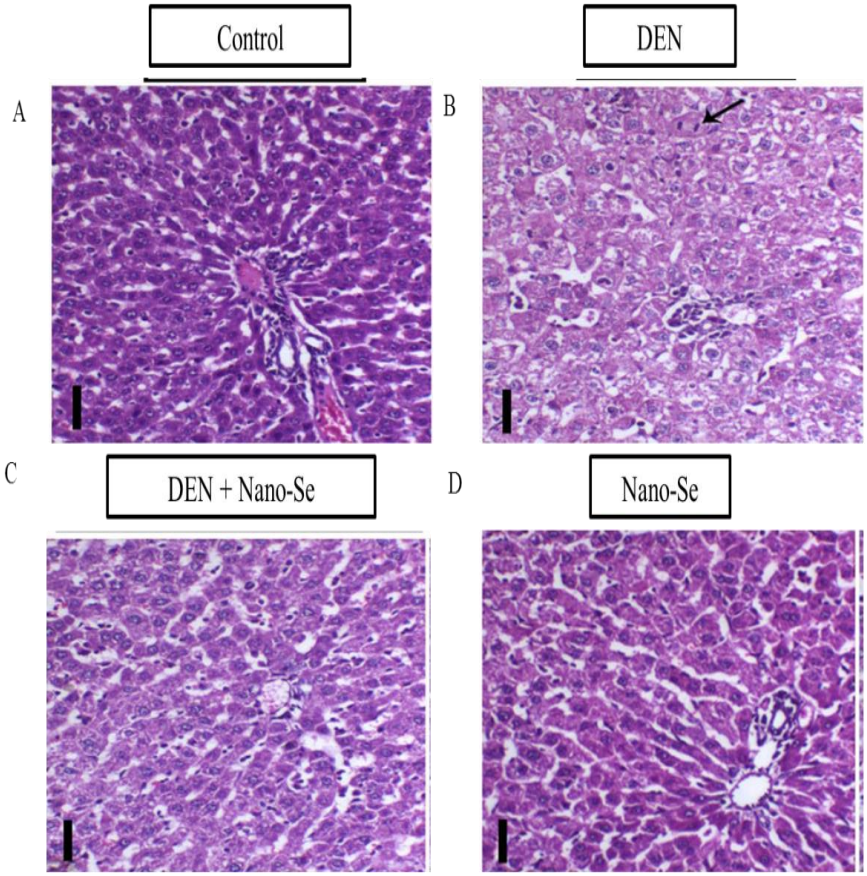
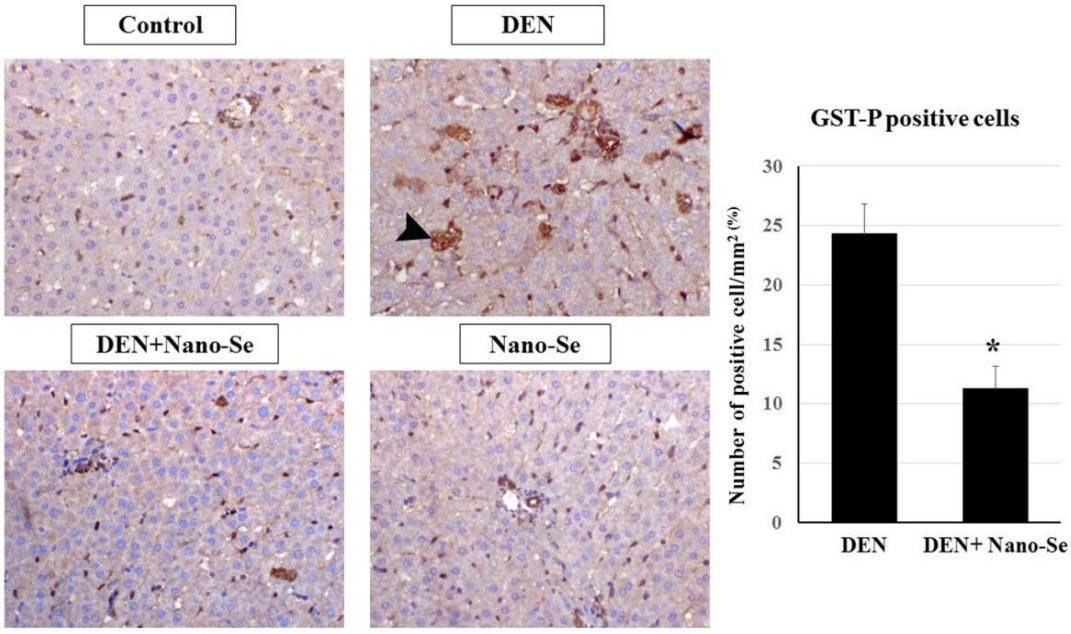


Figure 3. Histology of liver sections obtained from the rats of the different treatment groups: (A) Control group 1,liver showing normal hepatic structures. (B) DEN alone group 2, liver showing increase in mitotic figures (arrow). (C) DEN + Nano SE group 3 ,the liver was within normal limits associated with marked decrease in the mitotic figures ; and (D) Nano –Se alone group 4 ,liver also showing normal hepatic structures. H&E, bar= 50 µm DEN, diethylnitrosamine, Nano-Se, nano-selenium



DISCUSSION

Current experiment designed to elucidate the effect of biologically produced nano elemental selenium against the oxidative stress induced by DEN. DEN considers ROS-generating carcinogen which results in formation of preneoplastic foci. ROS generated inside the mammalian bodies are normally neutralized by the endogenous antioxidants (Ibrahim and Abdel-Daim 2015). Therefore, antioxidants consider the cellular housekeeper's through mopping up free radicals before they cause DNA damage.

The antioxidants may act as free radical scavengers, reducing and chelating agents for metals transition, quenchers of singlet oxygen molecules and activators of anti-oxidative enzymatic defense system that suppress free radical damage in biological systems (Abdel-Ghaffar 2013). Cancer in human and animals mostly arise from increased cellular oxygen-free radicals, which could induce direct chromosomal mutations and abnormalities causing a blockage of DNA replication (Valavanidis *et al.,* 2013).

Selective and efficient targeting of cancer cells is the foremost goal in the improvement of anti-cancer therapeutics (Pabla and Dong 2012). Biologically produced Nano-Se could be safer and acceptable than that produced by chemical process. Selenium considers an important part of the antioxidative system along with other antioxidants like vitamin E in the diets for prevention and/or treatment of vascular, muscular and hepatic lesions. Nano-particulate form of selenium has hepatoprotective and anticarcinogeneic effects (Zhang *et al.,*2004). Hematological parameters can be used to monitor interactions of chemicals with the biological system *In vivo* in order to assess their systemic effects.

In the present investigation, no alteration was detected on the erythrogram. However, DEN administration caused significant leukocytosis, marked neutrophilia, and monocytosis associated with with marked lymphocytopenia and eosinopenia compared with control group. DEN also significantly elevated MDA level with increased liver injury biomarkers as ALT, AST and GGT enzymes.

The association between neutrophilia and increase MDA associated with with depletion of antioxidant activity of CAT and GSH stores can be supported by finding of Amulic *et al.* (2012), who explained that the neutrophil-mediated pro-tumor mechanism through numerous inflammatory mediators, cytotoxic molecules and mitogenic factors. Neutrophils also induced oxidative DNA damage (Jaeschke 2006). These data was potentiated by the findings of Wilson *et al.* (2015) who correlated the juxtaposition of neutrophil rich inflammatory foci with hepatocytes positive for 8-hydroxyguanosine in human HCC tissue. Moreover, co-culturing of primary hepatocytes directly with neutrophils or indirectly with neutrophil-derived diffusible factors was sufficient to elevate the hepatocellular ROS levels.

Interestingly, Nano-Se ameliorates the adverse effect of DEN and return the different values of neutrophils, monocytes, lymphocytes and eosinophils to the normal limits. Experimental animals supplemented with selenium was associated with enhancement ofnatural killer cell activity, T-cell proliferation, lymphokine-activated killer cell activity, delayed-type hyper sensitivity skin responses, and vaccine-induced immunity (McKenzie *et al.,* 1998).

Meanwhile, selenium deficiency was accompanied with reduction of the lymphocyte reproductive potency especially in the animals with selenium deficiency (Pighetti *et al.,* 1998). Similarly, Lessard *et al.* (1991) believed that selenium played a role as an additive factor in enhancing the lymphocyte reproduction. Also, Kumar *et al.* (2008) noticed that Se had no effect on serum total proteins and albumin/globulin ratio, but could increase the capacity of the immune system to protect cells from free radical injuries.

The liver transaminase enzymes ALT and AST are considered markers for assessing liver function, elevated serum levels of these enzymes are indicating presence of hepatic cellular damages and loss of functional integrity of the cell membrane (Kumar *et al.,* 2005). Supplemention of Nano -Se alone didn't reveal any alteration in serum enzymes (Kumar *et al.,* 2008). While, DEN administration provoked marked elevation in serum ALT, AST and γGT activities which indicated hepatocellular injury.

The histopathological findings revealed centro-lobular hepatocytic injury which may be correlated with an increase in lipid peroxidation and more production of toxic aldehydes as MDA, as well as release the intracellular enzymes as ALT, AST and γ-GT associated hepatocellular injury (Nyblom *et al.,* 2006). In the current study, DEN-induced significant increase in MDA levels together with reduced antioxidant enzymatic system as catalase and glutathione. Glutathione considers one of the most abundant tri-peptide, biological non-enzymatic antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Decreased level of GSH is associated with an enhanced lipid peroxidation. Imbalance between production and neutralization of ROS results in serious disturbances in cells metabolism and induces oxidative damage to biomolecules, DNA, lipids and proteins (Eldahshan and Abdel-Daim 2015). DEN may initiates a series of mutagenic environment which lead to cancer.

An explanation for the oxidative stress associated with HCC induced in experimental animals may be attributed to the metabolic bioactivation of DEN by cytochrome p450 enzymes specifically CYP-(2E1) to ethyldiazonium ion which alkylates DNA bases to form promutagenic adducts such as O6-ethyldeoxyguanosine and O4 and O6-ethyldeoxythymidine. These reactive metabolites are primarily responsible for its hepatotoxic effects by inducing oxidative stress and cytotoxicity by damaging biomolecules such as DNA, lipids and proteins (Verna *et al.,*1996). Our results were consistent with El Mesallamy *et al.* (2011) who stated that DEN altered antioxidative defense as indicated by a significant elevation in the level of oxidative stress marker (MDA) and a significant impairment of free radical scavenging antioxidants (GR, GPx, SOD and GSH). Depletion in GSH level and GSH dependent enzymes, glutathione peroxidase (GPx) and glutathione reductase (GR) in DEN treated rats attributed to the reduction in their biosynthesis during hepatocellular damage or their excessive utilization in scavenging the free radicals formed during the metabolism of DEN. Additionally, the decreased levels of cellular GSH might have caused a reduction in the activities of GSH-dependent enzymes, GPx and GR, as GSH is a vital co-factor for these enzymes (Pradeep *et al.,* 2007). Nano-SE supplementation succeeded in restoring hepatic integrity by lowering oxidative stress, as the administration of Se nanoparticles caused a significant increase in antioxidant activities (GSH and CAT) and depletion of MDA level. Thus, counter act the DEN adverse effect on oxidative stress and antioxidant status. Our findings are in harmony with (Sadeghian *et al.,* 2012) who clarified that when Nano-selenium was administered to sheep as feed supplements, it reduced the levels of thiobarbituric acid reactive substances (TBARS) in plasma indicating a decrease in lipid peroxidation. Similarly, antioxidant effects of inorganic, organic, and elemental nano-selenium were studied in growing weaned Taihang black male goats in a 90-day experiment, higher antioxidative activities were observed in nanoparticle-treated animal compared to control (Shi *et al.,* 2011b). Similarly, improved antioxidant status were monitored when they were applied as dietary supplementation in a fish like crucian carp (Carassiusauratus Gibelio) (Zhou *et al.,* 2009). Treatment with Nano-Se prevented the depletion of antioxidant enzyme activity and restored the activities of these enzymes toward normal, thus, providing protection of bio-membrane from oxidative attack. Our observations are in line with reports of (Zarei and Shivanandappa 2013) who stated GSH depletion leads to lowering cellular defense against free radical-induced cellular injury resulting in necrotic cell death.

Also, our histopathological results showed marked increase in the neoplastic cell which usually showed clarification of their cytoplasm or marked eosinophilia which confirmed through positive GST-P expression within these cells. GST-P positive putative foci might be reveal the early carcinogenic effect induced by DEN administration. Nano-Se co-administration with DEN revealed significant decrease the neoplastic-GST-P positive cell, which could be indicate by decreasing the carcinogenic DEN-initiated cells. The mechanism of inhibition of cancer cell proliferation could be due to induction of apoptosis or cell cycle arrest or a combination of these both actions. (Luo *et al.,* 2012) noticed that nano-se arrested HeLa cells in the S phase, with further inhibition of mitosis and proliferation of cancer cells. On the same line, (Ahmed *et al.,* 2014) who detected that nano-se enhanced the necrotic/ apoptotic rate in liver tissues of HCC-rats.

Therefore, our result support that the hepatoprotective effect of Nano-Se against DEN-induced toxicity and hepatic damage involved inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels in addition to free radicals scavenging action. This could indicate the potent antioxidant activity possessed by elemental-Se. In addition to its ability to inhibit the proliferation in cancer cells through amelioration of the oxidative stress induced by DEN.

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جزيئات السيلينوم متناهيه الصغر المنتجه بيولوجيا تقلل مرحلة البدء في سرطان الكبد بالدایاثیل نیتروزامین

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تعتبر جزيئات السيلينوم متناهيه الصغر المنتجه بيولوجيا (نانو-سي) اقل سمية بالمقارنة مع الأشكال الأخرى من السيلينيوم وقد قيمت الدراسة الحالية التأثير الوقائي للنانو سيلينيوم المنتج بيولوجيا (نانو-سي) ضد التسمم الكبدي المبكر الذي يسببه ثنائي إيثيل نيتروزامين (دي اي ان) لمدة أسبوع واحد في الجرذان. تم تقسيم أربعين من ذكور الفئران إلى أربع مجموعات على النحو التالي: المجموعة ١ تعاملت بالحقن البرتوني (IP) بمحلول الملح الفسيولوجي مرة واحدة واحتفظ بها كمجموعة ضابطة، مجموعة ٢ تعاملت بمادة دي اي ان وحدها (٢٠٠ ملغ / كلغ) عن طريق الحقن البرتوني (I.p.) مرة واحدة. مجموعة ٣ تعاملت بكل من (د اي ان) ونانو سيلينيوم ومجموعة ٤ تعاملت بالنانو سيلينيوم وحده (٠.٥ ملغ / كلغ) عن طريق الفم. النتائج: اعطاء ال دا ايه ان لم يغير صورة كريات الدم الحمراء ولكن احدث تغييرات كبيرة في صورة كريات الدم البيضاء التي تمثلت في زيادة العدد الكلي لخلايا كريات الدم البيضاء والخلايا متعادلة الصبغة والخلايا وحيدة الانوية جنبا إلى جنب مع انخفاض كبير في اعداد الخلايا الليمفاوية ($P \leq 0.05$). ولوحظت سمية الكبد الناجمة عن ال (دي اي ان) من خلال ارتفاع الانزيمات الكبدية في الدم مثل أيه اس تي، أيه ال تي وجاما جلوتاميل ترانسفيريز. بالإضافة إلى ذلك، لوحظت زيادة في دلائل الاكسدة كمحتوى مالونديالدهيد (ام دي ايه) والحد من مؤشرات مضادة للأكسدة مثل الكاتاليز (كات) والجلوتاثيون المختزل (جي اس اتش). وعلاوة على ذلك، ارتبط (دي اي ان) مع تشكيل بؤر الكبد المفترضة المشار إليها مع زيادة التعبيرات الهستوكيميائية للجلوتاثيون ترانزفيراس شكل مشيمي جي اس تي-بي. ومن المثير للاهتمام، ان النانو-سي يخفف بشكل واضح من الآثار السلبية الناجمة عن سرطان الكبد لل د اي ان عن طريق عودة صورة كريات الدم البيضاء إلى حالتها الطبيعية، وقمع ارتفاع الانزيمات الكبدية في الدم، ومؤشرات الإجهاد التأكسدي، وانخفاض بؤر (جي اس تيبي) وزيادة ملحوظة في قدرة المضادة للأكسدة لذلك، يمكن أن نعتبر النانو سيلينيوم المنتج بيولوجيا كاستراتيجية فعالة في الوقاية الكيميائية لسرطان الكبد.