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Sodium selenite enhances glutathione peroxidase activity and DNA strand breaks in hepatoma induced by N-nitrosodiethylamine and promoted by phenobarbital

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Abstract An element/compound that acts as an antioxidant as well as, can increase the oxidative stress offers a new approach in differentiation therapy. Experiments were carried out to determine the effect of selenite on DNA damage and glutathione peroxidase (GPx) activity in N-nitrosodiethylamine (DEN) induced, phenobarbital promoted rat hepatoma. Supra-nutritional level of selenite (4 ppm) was supplemented at either, before-initiation/after-initiation and/or during entire period of the study. At the end of experiment period (20 weeks), extent of DNA damage (alkaline comet assay), selenium concentration, and GPx activity were assessed on nodular tissue (NL) cells, surrounding liver (SL) cells, and whole liver tissue (control) cells. Hepatic selenium level and GPx activity were decreased in DEN and PB-administered animals, whereas the DNA damage was found to be increased in both NL and SL cells compared with control group. However, the DNA damage is more in SL cells than in NL cells. Pre-supplementation of selenite did not show any difference in DNA (strand breaks) damage, selenium, and GPx activity. Increased hepatic selenium concentration and GPx activity were observed in both NL and SL cells in post-supplementation and entire period of selenite supplemented animals compared to DEN + PB treated animals. However, DNA damage was increased in NL but decreased in SL cells. Supplementation of selenite alone for 16 or 20 weeks had shown increased DNA damage, selenium concentration, and GPx activity compared to normal control animals. In summary, cancer bearing animals increased DNA damage and decreased Se level and GPx activity in NL and SL cells and other organs in cancer bearing animals, supplementation of Se further provoked DNA damage (no change in pretreatment) in NL cells, however it decreased DNA damage SL cells and other organs (kidney, lungs, and spleen). On the other hand Se levels and GPx activity were increased in NL and SL cells and other organs of Se-supplemented rats (no difference in group 3 animals). These results demonstrate that, in addition to chemopreventive and chemotherapeutic role of selenite, it also prevents cellular DNA damage induced in cancerous condition.

Keywords Selenite · Hepatoma · DNA damage · Comet assay

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Introduction

The primary goal in the prevention of cancer and other mutation-related diseases is the avoidance of exposure to recognized risk factors. Strengthening of the host defense mechanisms provides a complementary preventive approach, which is particularly important when targeted to

high-risk individuals. This strategy, referred to as chemoprevention, has found broad applications for the control of risk factors in cardiovascular diseases, and deserves greater emphasis in the prevention of cancer [1, 2]. The intake of protective factors can be achieved by means of both dietary measures and pharmacological agents.

The anticarcinogenic effect of selenium (Se) against various set of cancer-causing agents, including irradiation and carcinogens that form DNA adducts has been demonstrated in most organs examined in animal models [3, 4]. Several studies carried out in humans show an inverse correlation between Se intake and cancer incidence at several sites, including prostate, colon, lung, and breast [5–8]. Although the precise mechanism of Se anticancer activity remains to be determined, it is widely believed that multiple pathways are involved. It is at various stages of clinical development as a chemopreventive agent, demonstrating its ability to induce specific molecular perturbation associated with apoptosis and angiogenesis [9–12], by increasing phosphorylation of p53 mitogen-activated protein kinase, dephosphorylation of Akt and extracellular signal-regulated kinase 1/2, and PARP cleavage [9, 13, 14].

Various organic and inorganic Se compounds, generally considered to be antioxidants, produced mixed results when tested in animal models and human subjects. Among them, sodium selenite has been shown to be most effective in both in vitro and in vivo [4, 15–17]. Previous studies from our group have shown that sodium selenite treatment increases the overall antioxidant capacity of the hepatoma bearing animals [18, 19]. Recent studies demonstrate selenite, not only as an antioxidant, but possess oxidizing properties in the presence of specific substrates [20–22]. Nutritional essentiality of Se linked to the functional activities of several enzymes and proteins that contain Se, known as selenoproteins [21]. Several of these selenoproteins have antioxidant activities. Although the functions of most have not been determined, the effect of Se modulating the activity of these proteins could explain the possible mechanism by which Se might suppress the carcinogenesis. In the present investigation, we attempted to elucidate the possible role of sodium selenite as an in vivo antioxidant and/or oxidizing agent in chemical carcinogenesis.

In general, the indices used to measure DNA damage can be categorized into two subgroups. The first subgroup includes (i) single cell gel electrophoresis (the comet assay) [23] and (ii) terminal uridine nick end-labeling assay (TUNEL) [24]. The major advantages of these methods are that they directly measure/quantify DNA, are simple to perform, and focus on a single cell [25]. The second group comprising various biomarkers of DNA damage and repair, either in blood or urine samples, which indirectly measures/quantifies DNA damage and repair [26, 27]. Different reactive species react with different nucleic acid bases, for example, hydroxyl radicals react with all four nucleic acid bases whereas singlet oxygen reacts mainly with guanine [28].

In the present investigation, we employed Comet assay, which detects various forms of DNA strand breakage dependent on the pH of electrophoresis [29]. Under alkaline conditions ($pH > 13$), it detects single-strand breakage, double-strand breakage, excision repair site, and alkaline-labile sites [30]. Under neutral conditions, it mainly detects double-strand DNA breakage [31] and is therefore considered to be suitable for detection of DNA damage. The advantages of the comet assay for the detection of DNA damage are as follows: (a) it has higher sensitivity than the ladder assay [32] and TUNEL staining [33]; and (b) it can provide more specific information about the extent and heterogenity of DNA damage compared to TUNEL staining [34]. Based on hepatocarcinogeneic property and presence of various foods, we have chosen DEN as hepatocarcinogen initiator [4, 18, 19]. The purpose of the present study is essentially to determine the action(s) of selenite, an essential trace element that has shown a substantial inhibition on DEN-induced rat liver carcinogenesis. This may help us to further understand the inhibitory effect of selenite on the biochemical and biological aspects of DEN-induced and PB-promoted rat liver carcinogenesis.

Materials and methods

Animals and diet

Male, Wistar strain of albino rats, of age 6 weeks was used in these experiments. The rats were procured from Tamil Nadu Veterinary College, Chennai, India. They were fed with normal rat chow marketed by M/s.Hindustan Lever Limited, Mumbai, India and were provided with clean drinking water *ad libitum*. It was found that the rat chow used to feed our experimental animals contains 0.1 ppm of selenium, which is believed to satisfy the normal requirement of rats [35].

Chemicals and their sources

The following chemicals were purchased from the indicated sources: DEN, PB, bovine serum albumin, and sodium selenite from Sigma Chemical Co., (St. Louis, MO, USA). All other chemicals, including solvents, used were of high purity and analytical grade marketed by SD fine chemicals, Mumbai and Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Experimental design

The rats were divided into eight groups consisting of six animals in each (to study the mortality of experimental animals more numbers of rats were used). Liver tumors were induced in groups 2, 3, 5, and 7 with a single intraperitoneal injection of DEN at a dose of 200 mg kg^{-1} body weight in saline at the age of 10 weeks. Two weeks after DEN administration, the carcinogenic effect was promoted by the promoter, phenobarbital. Promoter was supplemented to the experimental animals through rat chow upto 14 successive weeks [18, 19].

Group 1 control animals were given the normal rat chow without additional selenite supplementation but the diet of groups 3, 4, 5, 6, 7, and 8 animals were supplemented with 4 ppm of selenium (as sodium selenite) in drinking water for various time periods as indicated below. Fresh drinking water supplemented with selenite was replaced on alternate days. The time point of DEN administration was taken as 0 (zero); minus (−) and plus (+) signs represents the time in weeks before and after DEN administration respectively. The schedule of selenium treatment in groups 3, 4, 5, 6, 7, and 8 was as follows: groups 3 and 4, −4 to 0; groups 5 and 6, +2 to +16; groups 7 and 8, −4 to +16. Groups, 4, 6, and 8 acted as selenium controls for groups 3, 5, and 7. The experiments were terminated 16 weeks after DEN administration (Fig. 1).

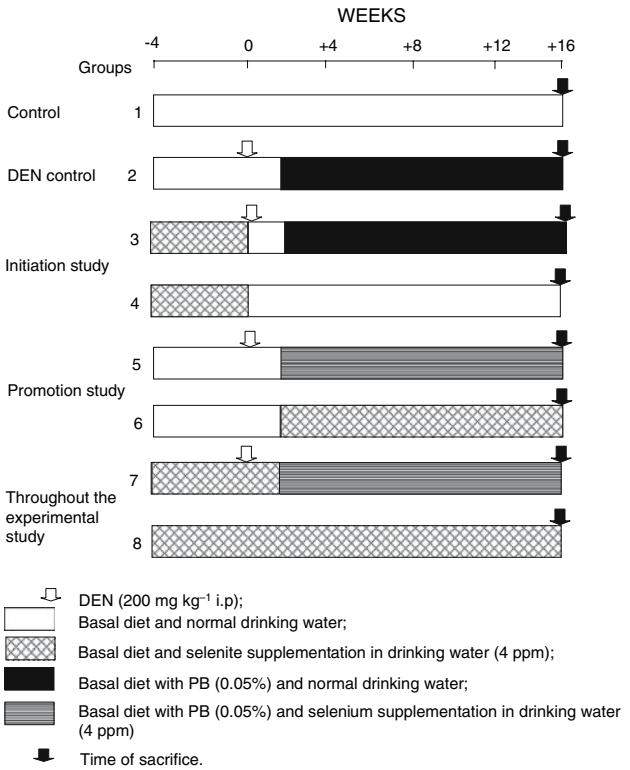


Fig. 1 Schematic representation of experimental regimen

Twenty weeks after the initiation of the experiment, all the experimental animals were fasted overnight and killed by cervical decapitation. Liver was perfused in situ with 0.15 M NaCl at 37°C. Blood was collected and serum was separated. Hyperplastic nodules and non-nodular surrounding liver tissues were obtained from all the groups treated with DEN. The greyish-white hyperplastic nodules were easily identified from the surrounding reddish-brown liver tissues. Tissue samples from spleen, kidney, and lung were also collected for analyses.

Comet assays

Comet assay was performed by the method of Dhawan et al. [36] with slight modifications [37]. Lysis solution (without sodium sarcosinate and with 10% DMSO—freshly prepared), Tris–HCl neutralization (0.4 M, pH 7.5) buffer and electrophoresis (EP) buffer (300 mM NaOH, 1 mM EDTA) were prepared as described by Singh et al. [37]. In brief, the tissues were sliced with fine scissors on ice with ice-cold PBS, and cells (1×10^4) were suspended in 110 µl of low melting point agarose (0.65% LMPA-w/v in PBS, pH 7.4) and pipetted onto a frosted glass microscope slide precoated with 140 µl of 1% normal melting point agarose (NMPA) (in PBS, pH 7.4). The agarose was allowed to set for 10 min at 4°C and thereafter, the cover slip was removed and the slides were exposed for 24 h to lysis solution. Finally, the slides were rinsed with distilled water and EP buffer to remove salts. These slides were exposed to alkaline EP buffer (pH 13.0) for 40 min, and subjected to EP for 20 min (300 mA, 25 mV). Then the alkali was neutralized with Tris–HCl buffer; the slides rinsed with distilled water and methanol, and were stained with ethidium bromide.

Slide scoring

Slides were scored using nebug, an image analysis system attached to a fluorescence microscope equipped with appropriate filters. The microscope was connected to a computer through a charge coupled device (CCD) camera to transport images to software for analysis. The final magnification was ×400, the parameters taken for the liver cells were: tail DNA (%), tail length (migration of the DNA away from the nucleus, µm), and tail moment (arbitrary units). Images from 100 cells (50 each replicate slide/10 randomly selected different field) were analyzed.

Biochemical investigations

Se concentration was determined by the fluorometric method of Olson et al. [38]. The activity of glutathione

peroxidase (GPx) was determined using hydrogen peroxide as substrate in the presence of reduced glutathione; estimation was carried out according to the method of Rotruck et al. [39]. The liver injury marker enzymes such as aspartate aminotransferase, lactate dehydrogenase, and γ -glutamyl transpeptidase were measured according to method of King [40, 41], and Massey and Williams [42] respectively. Total protein and albumin was estimated by the method of Reinhold [43]. For some biochemical assays, nodular or surrounding tissues were pooled together from different animals of the same group to get enough amounts of tissues.

Statistical analysis

Statistically significant ($P < 0.05$) differences between different groups were done using ANOVA and Student's *t*-test. Each value in the results section represents two-way significance tests, i.e., b, represents significance against group 2 DEN-control and a, c, d, and e represents the same against their respective controls (groups 1, 4, 6, and 8).

Results

Food and water intake

During our experimental period, no differences in food and water consumption were observed between the different groups of animals. Food and water intakes were 11.5–14.6 g of diet/day/100 g of body weight and 8.5–11.5 ml of water/day/rat, respectively. A total of two rats from group 2 (16.6%) died before the end of the study. None of the rats from any other group died during the experimental period.

Changes in body weight and weights of organ

Table 1 shows the final body weight, liver, spleen, lung, and kidney of different groups of rats that were killed after 20 weeks of the study. The final body weight of DEN control rats (group 2) was significantly less ($P< 0.01$), where as liver weight is increased than that of the normal vehicle control (group 1). Supplementation of 4.0 ppm selenium for 20 consecutive weeks maintained the body weight at normal level and there were no significant differences between group 1 (normal vehicle control) and group 8 (selenium control) suggesting that selenium supplementation in this study did not have any adverse effect on the growth responses of the rats. Treatment with selenium for 20 weeks significantly increased ($P< 0.05$) the final body weight and reduced the liver weight of group 7 rats compared to the carcinogen control (group 2). There was no significant difference among the groups in their liver, spleen, lung, and kidney weights.

Se and GPx activity

Table 2 shows the hepatic concentration of Se and GPx activity. The Se level and GPx activity are significantly decreased in NL and SL tissue of carcinogen bearing animals (group 2) when compared to group 1 animals. Pre-supplementation (group 3) of Se did not show any difference in Se levels and GPx activity when compared to group 2 animals. On the other hand post-supplementation and throughout the study, Se-treated groups (group 5 and 7) show increased level of Se and GPx activity. A significant difference in both Se and GPx activity was observed in both NL and SL tissues (except group 3) as compared with the values of the carcinogen (group 2) control rats and also with their respective controls, viz. groups 6 and 8.

Table 1 Effect of sodium selenite on body weight, liver weight, and weight of different experimental groups ($n = 8$ for pair feed control animals and $n = 12$ for experimental groups)

Groups	Number of rats	Final body weight (g)	Liver weight (g)	Spleen weight (g)	Lungs weight (g)	Kidney weight (g)
1	8/8	315 ± 22.6	10.09 ± 2.36	0.52 ± 0.09	1.84 ± 0.2	3.24 ± 0.3
2	10/12	268 ± 22.3a$^{@}$	14.21 ± 2.31a*	0.41 ± 0.30	1.80 ± 0.3	2.98 ± 0.4
3	12/12	272 ± 26.2	13.1 ± 1.98	0.48 ± 0.36	1.95 ± 0.4	3.02 ± 0.4
4	8/8	326 ± 32.5	11.0 ± 1.5	0.63 ± 0.23	1.94 ± 0.3	3.35 ± 0.4
5	12/12	278 ± 31.5	12.93 ± 1.68	0.51 ± 0.35	2.03 ± 0.5	3.12 ± 0.3
6	8/8	309 ± 30.6	10.08 ± 1.9	0.56 ± 0.38	2.02 ± 0.4	3.25 ± 0.3
7	12/12	296 ± 30.5b*	11.02 ± 2.01b*	0.59 ± 0.61	2.08 ± 0.4	3.15 ± 0.5
8	8/8	319 ± 25.6	11.02 ± 2.13	0.68 ± 0.48	2.16 ± 0.5	3.32 ± 0.4

Groups were treated as mentioned in section Materials and methods

Each value represents mean ± SEM. Significance from; "a" as compared with group 1; "b" as compared with group 2

* $P < 0.05$, $^{@}$ $P < 0.01$

Table 2 Changes in the total hepatic selenium concentration and glutathione peroxidase activity in whole liver tissue in control, and in nodular tissue (NL) and surrounding liver tissue (SL) experimental animals

Particulars		Se concentration ng/g wet tissue	GPx activity μg of glutathione utilized/min/mg protein
1	Whole liver tissues (Control)	0.62 ± 0.08	86.6 ± 9.0
2	Hepatoma (NL)	0.31 ± 0.02a$^{\#}$	36.9 ± 2.9a$^{\#}$
	Surrounding (SL)	0.36 ± 0.02a$^{\#}$	44.8 ± 3.5a$^{\#}$
3	Hepatoma (NL)	0.35 ± 0.03b^{NS}c$^{\#}$	41.5 ± 3.1b^{NS}c$^{\#}$
	Surrounding (SL)	0.38 ± 0.03b^{NS}c$^{\#}$	58.6 ± 5.0b$^{\#}$c$^{\#}$
4	Whole liver tissues (Control)	0.61 ± 0.11	88.7 ± 8.3
5	Hepatoma (NL)	0.39 ± 0.03b$^{@}$d$^{\#}$	47.8 ± 4.7b$^{\#}$d$^{\#}$
	Surrounding (SL)	0.43 ± 0.03b$^{@}$d$^{\#}$	63.2 ± 4.9b$^{\#}$d$^{\#}$
6	Whole liver tissues (Control)	0.69 ± 0.09	91.9 ± 5.5
7	Hepatoma (NL)	0.47 ± 0.02b$^{\#}$e$^{\#}$	73.9 ± 8.5b$^{\#}$e$^{@}$
	Surrounding (SL)	0.51 ± 0.03b$^{\#}$e$^{@}$	78.7 ± 7.5b$^{\#}$e$^{@}$
8	Whole liver tissues (Control)	0.75 ± 0.11a*	95.7 ± 10.3a*

Groups were treated as mentioned in section Materials and methods

Each value represents mean ± SEM ($n = 6$); "a" as compared with group 1; "b" as compared with group 2; "c" as compared with group 4; "d" as compared with group 6; "e" as compared with group 8

* $P < 0.05$, $^{@}$ $P < 0.01$, $^{\#}$ $P < 0.001$, ^{NS}not statistically significant

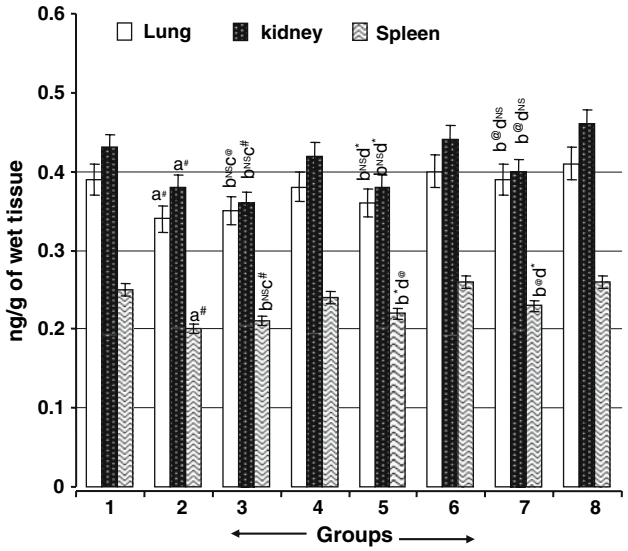


Fig. 2 Levels of selenium in different experimental groups (details see Materials and methods). Each value represents mean ± SEM ($n = 6$); "a" as compared with group 1; "b" as compared with group 2; "c" as compared with group 4; "d" as compared with group 6; "e" as compared with group 8; (* $P < 0.05$, $^{@}$ $P < 0.01$, $^{\#}$ $P < 0.001$, ^{NS}not statistically significant)

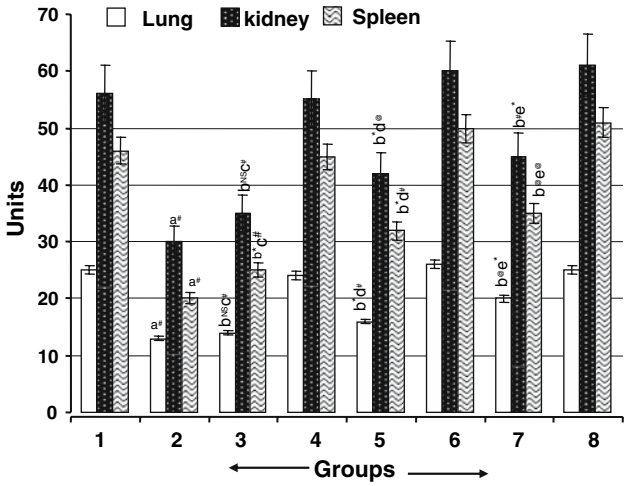


Fig. 3 GPx activity in control and experimental animals. Groups were treated as mentioned in section Materials and methods. GPx activity is expresses as unit, one unit corresponds to μg of glutathione utilized/min/mg protein at 37°C, each value represents mean ± SEM ($n = 6$); "a" as compared with group 1; "b" as compared with group 2; "c" as compared with group 4; "d" as compared with group 6; "e" as compared with group 8; (* $P < 0.05$, $^{@}$ $P < 0.01$, $^{\#}$ $P < 0.001$, ^{NS}not statistically significant)

Se level and GPx activity in lung, kidney, and spleen of different experimental groups are shown in Figs. 2 and 3. In the carcinogen control group (group 2), the GPx activity and Se level were found to be lower when compared with normal control animals (group 1). Se level and GPx activity are liable towards the normal value upon Se supplementation.

Liver function test

The synthesizing capacity of the liver was reduced in carcinogen + promoter alone (group 2) treated rats, as indicated by decreased serum albumin, which was normalized by Se treatment for 20 weeks (group 7; Fig. 4a and b). Se treatment before initiation (group 3) and during promotion (group 5) also

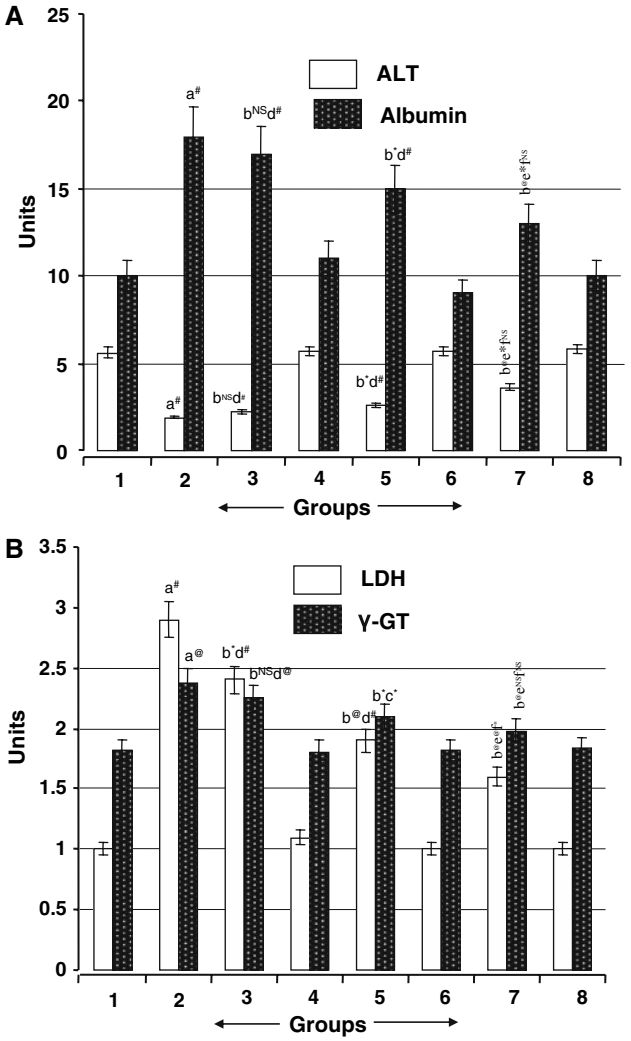


Fig. 4 Level of albumin and ALT activity (**a**) and activities of LDH and γ-GT (**b**) in serum of control and experimental groups. Albumin level was expressed as unit, one unit corresponds to mg/dl of serum. ALT, LDH and γ-GT activities are expressed as units, for ALT and LDH one unit corresponds to µmoles of pyruvate liberated/minute/mg of protein at 37°C, for γ-GT one unit corresponds to nmoles of p-nitroaniline formed/minute/mg of protein at 37°C. Groups were treated as mentioned in section Materials and methods. Each value represents mean ± SEM ($n = 6$); "a" as compared with group 1; "b" as compared with group 2; "c" as compared with group 4; "d" as compared with group 6; "e" as compared with group 8; "f" as compared with group 7; (* $P < 0.05$, $^{@}$ $P < 0.01$, $^{\#}$ $P < 0.001$, ^{NS}not statistically significant)

increased liver function 16.9% and 28.7% respectively. Liver injury, as estimated by serum LDH, AST, and α-GT, also improved significantly in group3 (∼18.29%), group 5 (∼34.38%), and group 7 (∼68.2%) Se-treated rats.

DNA damage

Figure 5 shows the DNA damage such as tail length (Fig. 6a), tail moment (Fig. 6b), % tail DNA (Fig. 6c) in control, and experimental animals. The carcinogen + promoter administered animals (group 2) the DNA damage is increased in NL ($P < 0.001$) and SL cells ($P < 0.001$) when compared with normal control group (group 1). SL cells showed more DNA (20%) damage than NL, if comparison made within group (group 2). Se supplementation for 4 weeks alone (before initiation of cancer-group3) did not show any statistical difference in DNA damage compared with carcinogen-treated group (group 2). However, the chemopreventive effect is not ruled out as reported by us earlier [18] and as shown in the present study. When Se was supplemented during promotion period (group 5), the DNA damage is increased in NL cells ($P < 0.001$), where as SL cells decreased ($P < 0.01$) when compared to group 2 animals. Group 6 animals compared with group 1 animals DNA damage is increased ($P < 0.05$). Se supplemented for 20 weeks, shown (group 7) significantly increased ($P < 0.001$) DNA damage in NL cells, whereas SL cells DNA damage was reduced ($P < 0.001$) when compared with group 2 animals. We did not observe any change (such as DNA laddering etc.) in DNA on agar gel electrophoresis and caspase-3 like activity (data not shown). This shows that there is no apoptotic-mediated cell death up on Se administration.

We measured DNA damage in lung, kidney, and spleen of control and experimental groups (Tables 3 and 4). In cancer bearing animals the DNA damage was increased considerably in lung, kidney, and spleen ($P < 0.05$; $P < 0.01$; $P < 0.01$, respectively). Se supplementation either before initiation alone (group 3) or during promotion alone (group 5) had no effect on these organs. Whereas Se supplementation for 20 weeks (group 7) reduced their DNA damage in lung ($P < 0.05$), kidney ($P < 0.01$) and spleen ($P < 0.01$). When pair fed control animals (group 4, 6, and 8) compare with normal control animals (group 1), there is no change in group 4 animals; but group 6 showed an increase in DNA damage in kidney and spleen, and group 8 showed an increase in kidney, spleen, and lung (all at $P < 0.05$).

Discussion

Previous studies from our laboratory demonstrated the chemopreventive and chemotherapeutic role of selenite, by increasing the oxidative defense molecules; which may be one of the mechanisms observed in multi-stage carcinogenesis [18, 19]. To further elucidate the possible in vivo mechanism of sodium selenite in hepatocarcinogenesis, we performed the present experiments. The extent of DNA damage (by comet assay) was measured as an indicator of oxidative stress. In addition, selenium and selenium-associated enzyme such as GPx was measured in the nodular,

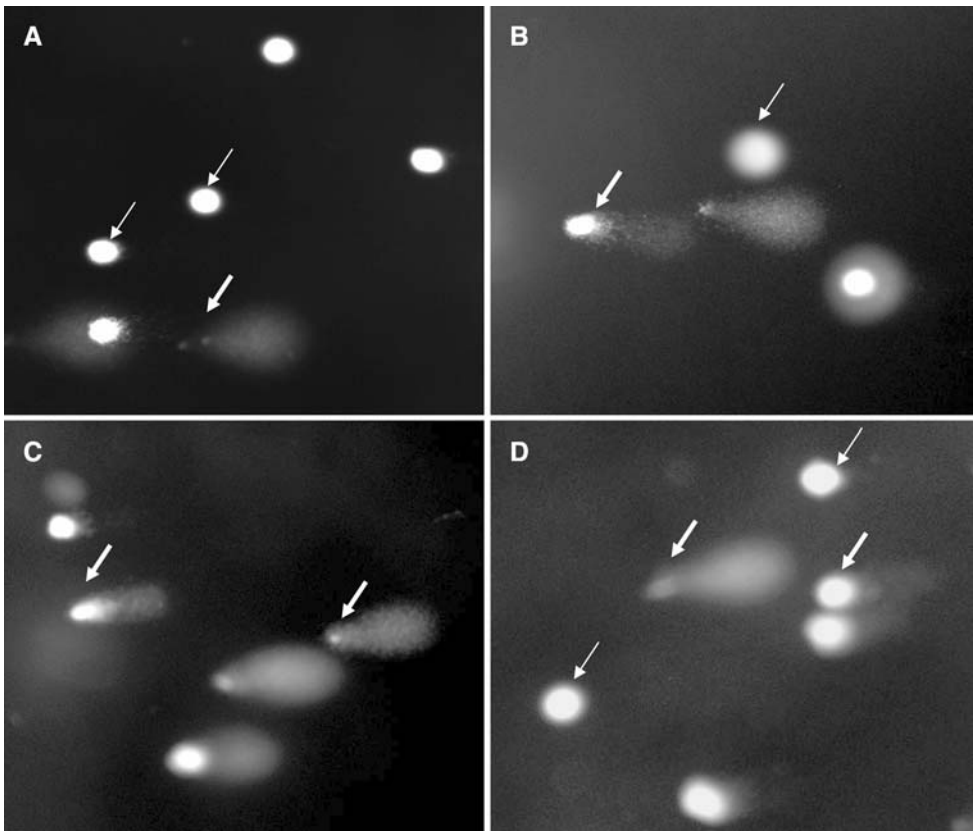
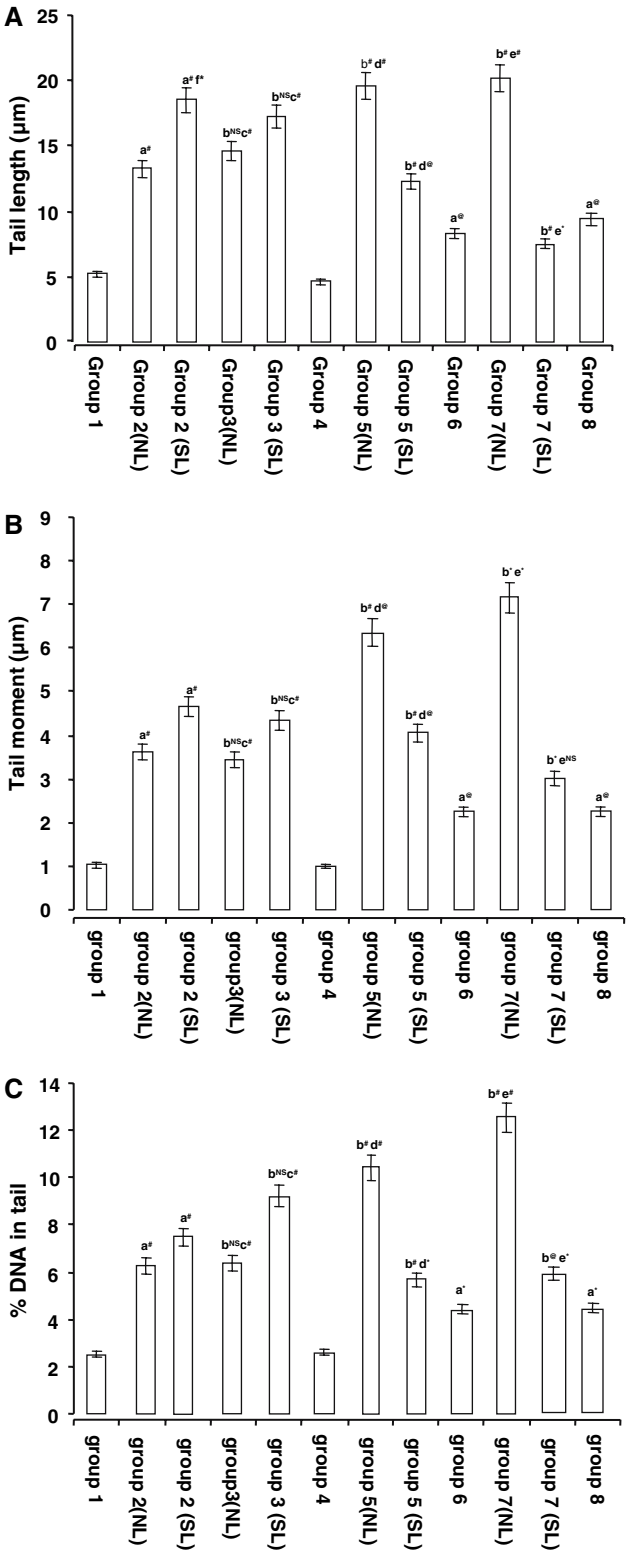


Fig. 5 Effect of sodium selenite on DNA damage in control and experimental groups. Control (**a**) and selenite alone treated animals (**d**) for 20 weeks whole liver cells were used, carcinoma-bearing animals (**b**) and selenite (20 weeks) + carcinoma-bearing animals (**c**) nodular tissue cells were used. Thin arrow head shows non-DNA damaged cells; thick arrow head shows DNA damaged cells

surrounding, and normal liver tissues of control and experimental groups. These parameters were employed based on the nature of anticancer drugs like cisplatin and mitomycin C, which are known to induce DNA damage in cancerous tissues; on the other hand antioxidant compounds such as quercetin and curcumin, which are also known for the anticancer effect through their antioxidant properties. However, to date as far as selenite is concerned, varying and conflicting results were reported [17, 44–46]. Hence, to understand the possible mechanism of action of selenite in vivo, we supplemented selenite either before initiation (group 3), during promotion (group 5), and through entire period of this study (group 7).

We observed reduction in the tissue selenium level, GPx activity, and increase in DNA damage in cancer-bearing animals not supplemented with Se. The increased DNA damage observed in cancer cells might be due to the reduced antioxidant capacity observed in cancerous animals [18, 19] or alteration in mineral content in cancer animals [47], for example: elevations in hepatic level of Fe^{2+}, iron, though vital in life-processes, is also potentially toxic to living cells due to its ability to exist in two stable and inter-convertible redox-active states, since redox reactions catalyze the formation of oxyradicals generating superoxide radicals (O_2^-), which is the precursor of toxic H_2O_2 [48]. Moreover, ferrous iron can reduce copper to the cuprous state, which is a more potent generator of hydroxyl radicals than ferrous ions, thus iron acts synergistically with copper in the carcinogenic process. Thus, antioxidant-deficient environment and accumulated free radicals ultimately favor DNA lesions resulting in increased hepatic cell proliferation, phenotypic transformation, and expression of neoplastic pathology with minimal apoptotic events. Se-mediated restoration of hepatic levels of antioxidant [18] may have a role in the repair of DNA base-lesions in vivo. Furthermore, normalization of hepatic Fe levels [48] minimizes the possibility of free-radical generation, thereby preventing oxidative injury to cells and DNA. Restoration of antioxidant level after treatment with Se has been linked with suppression of cell proliferation events [49]. Studies from our laboratory indicate that, at a dose of 4.0 ppm increases the antioxidant levels and maintains membrane integrity [18, 50].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated as a consequence of carcinogen exposure, leading to DNA-strand breaks [51]. For the cell, double-strand breaks (DSBs) are probably the most deleterious form of DNA damage and may arise during the replication of single strand breaks (SSBs), when carcinogen-induced SSBs remain unrepaired [52]. Error-prone repair of DSBs can lead to chromosomal aberrations as well as oncogene activation, which contribute to carcinogenesis. Thus, SSBs can be considered as a fundamental to the maintenance of chromosome integrity and genetic



◀**Fig. 6** Effect of sodium selenite on tail length (**a**), tail moment (**b**) and percent tail DNA (**c**) of whole liver tissue cells, nodular tissue (NL) and surrounding liver tissue (SL) cells of control and experimental animals. Groups were treated as mentioned in section Materials and methods. Each value represents mean ± SEM ($n = 6$); "a" as compared with group 1; "b" as compared with group 2; "c" as compared with group 4; "d" as compared with group 6; "e" as compared with group 8; "f" compared with group 2 NL Vs group 2 SL; (* $P < 0.05$, $^{@}$ $P < 0.01$, $^{\#}$ $P < 0.001$, ^{NS}not statistically significant)

stability. In the present study, a substantial decrease in the amount of DEN-induced 'tailed' DNA and DNA 'comets' by Se could reflect its anticlastogenic potential to combat genotoxicity. Selenite reacts with reduced form of glutathione (GSH) in the metabolic process leads to the formation of ROS, (see below).

Another potential mechanism of Se that comes out from our study is Se-mediated induction of DNA damage particularly in cancer cells. The involvement of free radical in many degenerating diseases including cancer based on the detection of the oxidation products of nucleic acids, proteins, and lipids formed as a consequence of diseased condition. Tumor growth is associated with tissue hypoxia that is accompanied by the formation of reductive rather than oxidative free radicals. Although, the most biologically active oxidant such as hydroxyl radical has been generated during hypoxia [53, 54]. Possible mechanism by which selenite causes more DNA damage in cancerous cells compared to surrounding cells is that cancer cell membrane-bound proteins are associated with polythiols, which appear under the reducing conditions of hypoxic tumor cells. These thiol groups can, in turn, initiate a disulfide exchange reaction with plasma proteins, predominantly with fibrinogen, to form an insoluble and protease-resistant fibrin-like polymer. As the result, tumor cells become surrounded by a coat that masks specific tumor antigens thus allowing cancer cells to escape immune recognition and elimination by natural killer cells [55–57]. Selenite is capable of oxidizing polythiols to corresponding disulfides, but does not react with monothiols. Selenite by virtue of oxidizing cell membrane thiols, can prevent the formation of the coat and consequently makes cancer cells vulnerable to the immune surveillance and destruction [55, 56].

Reduced glutathione is involved in Se metabolism and its bioactivity. Previous study from our group found that cancer cells have low levels of reduced glutathione [19]. Study by Shen et al. [25] shows that both increase and depletion of reduced glutathione content enhances the selenite-induced oxidative stress and apoptosis in human hepatoma cells. It should be pointed out, that there are two fundamental differences between the group 5 versus group 3. Pretreatment of selenite for 4 weeks (group 3), inhibited tumor incidence around ~25% where as group 5

Table 3 Tail length, tail moment, and percent tail DNA in kidney of control and experimental groups. Groups were treated as mentioned in section Materials and methods

Particulars	Tail length	Tail moment	% Tail DNA
Group 1	1.08 ± 0.25	0.36 ± 0.06	5 ± 2
Group 2	3.45 ± 0.45a$^{\#}$	1.56 ± 0.26a$^{\#}$	25 ± 4a$^{\#}$
Group 3	2.86 ± 0.23b^{NS}c$^{\#}$	1.44 ± 0.24b^{NS}c$^{\#}$	26 ± 6b^{NS}c$^{\#}$
Group 4	1.02 ± 0.15	0.32 ± 0.04	4 ± 2
Group 5	2.25 ± 0.35b^{NS}d$^{@}$	1.40 ± 0.35b^{NS}c*	18 ± 5b*c*
Group 6	2.01 ± 0.24a*	0.89 ± 0.09a*	9 ± 3
Group 7	1.56 ± 0.32b$^{@}$e^{NS}	0.75 ± 0.06b$^{\#}$e^{NS}	12 ± 4b*e^{NS}
Group 8	2.02 ± 0.25a*	0.99 ± 0.09a*	9 ± 4

Groups were treated as mentioned in section Materials and methods

Each value represents mean ± SEM ($n = 6$); "a" as compared with group 1; "b" as compared with group 2; "c" as compared with group 4; "d" as compared with group 6; "e" as compared with group 8

* $P < 0.05$, *** $P < 0.01$, ** $P < 0.001$, ^{NS}not statistically significant

Table 4 Tail length in lung and spleen of control and experimental groups

Particulars	Spleen	Lung
Group 1	0.82 ± 0.07	0.66 ± 0.08
Group 2	2.26 ± 0.26a$^{\#}$	2.02 ± 0.31a$^{\#}$
Group 3	2.12 ± 0.19b^{NS}c$^{\#}$	2.01 ± 0.22b^{NS}c$^{\#}$
Group 4	0.80 ± 0.06	0.68 ± 0.05
Group 5	1.96 ± 0.02b^{NS}d$^{@}$	1.82 ± 0.02b^{NS}d$^{@}$
Group 6	1.07 ± 0.11a*	0.89 ± 0.07
Group 7	1.56 ± 0.02b*e*	1.25 ± 0.01b*e*
Group 8	1.09 ± 0.13a*	0.91 ± 0.01a*

Groups were treated as mentioned in section Materials and methods

Each value represents mean ± SEM ($n = 6$); "a" as compared with group 1; "b" as compared with group 2; "c" as compared with group 4; "d" as compared with group 6; "e" as compared with group 8

* $P < 0.05$, $^{@}$ $P < 0.01$, $^{\#}$ $P < 0.001$, ^{NS}not statistically significant

(16 weeks of post-treated selenite) shows only ~15–18% inhibition [4]. However, there is no difference in the extent of DNA damage in group 3 but group 5 showed increased DNA damage in cancer cells. Selenite post-treated group showed further increase in DNA damage with minimal antitumor activity, these results shows that sodium selenite play a dual role in chemical carcinogenesis. For example, short time supplementations of Se in normal animals increase the intracellular GSH levels [58, 59]. In vitro studies show controversial reports on the changes in intracellular GSH [60–62]. We believe, short-time supplementation of selenite might increase some of the selenium-related proteins and/or GSH [58, 59]. Increased GSH may reduce the carcinogen-DNA interaction or increase carcinogen metabolism, which in turn reduce tumor incidence. Continuous supplementation of Se may result in the generation of oxidative stress, which has been proposed as one of the mechanisms by which this element exerts its cellular actions in cancer cells.

Selenium potentially affect cancer development through its oxidative stress, DNA repair, inflammation, apoptosis, proliferation, carcinogen metabolism, testosterone production, angiogenesis, fat metabolism, and immune function [13, 44, 63, 64]. Natural organic (e.g., selenomethionine) and inorganic (e.g., selenite) forms of Se are metabolized via different pathways into selenide, which then be either phosphorylated and ultimately incorporated as selenocysteine into active selenoproteins or methylated into active metabolites, such as methylselenol [65, 66]. Therefore, the effect of Se can be indirect (via incorporation into selenoproteins) and/or direct (via selenium metabolites). Direct effect of Se varies with different metabolites, in normal versus malignant prostate cells. The most active known metabolites in preclinical studies are natural methylated compounds (e.g., methylselenol) and synthetic organoselenium compounds (e.g., 1,4 phenylenebis(methylene) selenocyanate) [65, 66]. The molecular targets include manganese superoxide dismutase, p21, caspase-8, NF-κB, protein kinase C, and the androgen receptor in prostate cancer [17, 44, 67–71]. Selenium indirectly effects via enzymatic functions of certain selenoproteins. Besides their well-known effects (e.g., of glutathione peroxidase) on intracellular redox, selenoproteins posses other activities, which varies with cell type, physiologic status, presence or absence of incorporated selenocysteine. For example, selenoprotein thioredoxin reductase without (but not with) selenocysteine appears to induce apoptosis and inhibit growth in certain cell types [72, 73].

Results from our present study demonstrate that sodium selenite increases DNA damage, selenium level, and GPx activity in NL cells, whereas decreases the DNA damage in SL cells and other organs of cancer-bearing animals. We also found that long-term supplementation of Se (group 6 and 8) alone causes DNA damage. On the other hand, these animals did not show any harmful effect such as weight loss, reduced food intake and liver toxicity. To elucidate exact mechanism(s) that how Se increases DNA damage in NL cells and decreased in SL cells further studies are needed.

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