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Selenium Supplementation Reduced Oxidative Stress in Diethylnitrosamine-induced Hepatocellular Carcinoma in Rats

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Abstract: Selenium in the form of sodium selenite (SSE) is an essential micronutrient which known to possess antioxidant and anticancer properties. This study emphasizes the role of selenium on oxidative stress in experimental rats with N-diethylnitrosamine (DEN) initiated and 2-acetylaminofluorene (2-AAF) promoted multistage hepatocellular carcinogenesis (HCC). Rats were divided randomly into six groups: negative control, positive control (DEN+2-AAF), preventive group (pre-SEE 4 weeks+DEN), preventive control (respective control for preventive group), therapeutic group (DEN+post-SSE 12 weeks) and therapeutic control (respective control for therapeutic group). SSE (4 mg L^{-1}) was given to animals before initiation and during promotion phase of HCC. The levels of total protein (TP), conjugated diens (CD), malondialdehyde (MDA), fluorescent pigment (FP), antioxidant activity (AOA) and DNA damage were measured. Supplementation of SSE before the initiation phase of carcinogenicity significantly increased TP and AOA level ($p<0.05$) while it decreased the levels of CD, MDA, DNA damage and FP ($p<0.05$). Supplementation of SSE during the promotion phase of carcinogenicity significantly decreased the DNA damage and FP level ($p<0.05$) and there were negative correlation between the level of AOA and with the level of FP and CD. Thus, supplementation of SSE reduced the adverse changes which occur in liver cancer. However, the chemoprevention effect of SSE was more pronounced when it was supplemented before initiation phase of cancer when compared to promotion phase.

Key words: Sodium selenite, diethylnitrosamine, 2-acetylaminofluorene, liver cancer, antioxidant

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of liver cancer, generally leading to death (Alkahtani, 2009; Hanachi *et al*., 2006). It is occurring with increasing frequency especially in the United States, Japan and it accounts for about 85-90% of primary liver cancers (Schafer and Sorrell, 1999). HCC occurs as a result of a malignant transformation in the epithelial cells of the hepatocytes. Exact molecular mechanism involves in cellular transformation and cancer cell development still unclear (Halliwell, 2002; Klaunig and Kamendulis, 2004). However, these condition attribute to a diverse range of causes and one of the most prominent cause beside hepatitis C virus and alcoholic consumption is oxidative stress (Sasaki, 2006; Farazi and DePinho, 2006; Alkahtani, 2009). Oxidative stress is manifested by an imbalance between the production of reactive oxygen (ROS) species and the respective defense systems in the biological system (Sulaiman *et al*., 2006). Previous researches suggested that increased oxidative stress plays important role in carcinogenesis (Oberley, 2002; Muslim *et al*., 2010) and pathogenesis of various diseases (Mantovani *et al*., 2002).

In order to maintain cellular health, antioxidant therapy has been focus as a remedy to treat and protect from the damages caused by the oxidative stress. SSE is a component of glutathione peroxidase (GPx), the potent antioxidant which detoxifies the oxygen radicals and peroxides. Selenodiglutathione, the metabolite of selenite, has the ability to specifically inhibit protein synthesis and growth of tumor cells (Vernie *et al*., 1974; Lanfear *et al*., 1994). Low concentrations of selenium in the diet may increase the risk of cancer (Rayman, 2000; Ghazi-Khansari *et al*., 2005; Avci *et al*., 2005; Jahan *et al*., 2011). However, there is still lack of evidences to recommend the use of selenium supplements for cancer prevention. Therefore, this study attempts to characterize

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the role of selenium on oxidative stress against experimentally induced HCC in rats by investigating the important biochemicals parameters for lipid peroxidation and antioxidant activity.

MATERIALS AND METHODS

This study was carried out in the Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia between April 2008 to 2010.

Animals and diets: Forty eight male, Sprague-Dawley (SD) rats (4 to 6 weeks old; 250 to 300 g weight) obtained from the Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, were used for the study. The rats were housed in plastic cages (2 rats/cage) in a temperature- and light-controlled environment and wood chips for bedding. Animals were allowed free access to tap water and food. Upon arrival, they were allowed to acclimatize for a week prior to the experiments. This project was approved by the Ethical Committee of University Kebangsaan Malaysia (UKMAEC No.: FSKB/2006/Jamaludin/22-August/170-December-2006).

Experimental design: The rats were divided randomly into six groups, each consisting of eight animals. Group 1; (negative control) given normal saline (0.9%). Groups 2; (positive control) administered with N-nitrosodiethylamine (DEN) (200 mg kg^{-1} b.w, single i.p.). Two weeks after administration of DEN, 2-AAF (0.02%) was incorporated into rat chow for up to 14 successive weeks to promote the cancer. Group 3; (preventive group) given SSE for 4 weeks (4 mg L^{-1} through drinking water) before the administration of DEN and followed by 2-AAF as in Group 2. Group 4; (preventive control) given SSE for 4 weeks. Group 5; (therapeutic group) administered with DEN (200 mg kg^{-1} bw, single i.p.) followed by SSE for 12 weeks and finally group 6; (therapeutic control) given SSE for 12 weeks. After 16 weeks, animals were fasted overnight and killed by cervical dislocation under ether anesthesia. Liver tissues were taken out for the biochemical investigation purpose (DEN, Sigma Chemical Company, USA).

Biochemical studies: Fresh liver tissues were homogenated according to the method of Stocks *et al.* (1974). Standard procedures were used to assay the following biochemical measurements. TP was measured by the method of Bradford (1976), using bovine serum albumin as a standard. CD was measured spectrophotometrically according to method of Recknagel and Glende (1984) with slight modification. MDA was measured colorimetrically by the method of Stocks and Dormandy (1971). Fluorescent pigment was measured fluorometrically by the method of Dillard and Tappel (1971). AOA was measured colorimetrically according to the method of Stocks *et al.* (1974) and Hunter and Mohamed (1986). Measurement of DNA damages was carried out using comet assay established by Singh *et al.* (1988).

Statistical analysis: Mean and Standard Error of Mean (SEM) were calculated for each parameter. Data were tested for parametric post hoc analyses and then analyzed by one-way analysis of variance (ANOVA) using SPSS 15.0 software (Chicago, IL, USA) and $p<0.05$ was considered to be statistically significant.

RESULTS

The effect of SSE on the levels of TP, CD, MDA, FP, AOA and the degree of DNA damage in the liver of control and experimental groups are summarized in Fig. 1-6. Generally, there were no significant different observed among the negative control, preventive control and therapeutic control groups for TP concentration ($p>0.05$) (Fig. 1). It is noted that the levels of TP significantly decreased in positive control group compared to the negative control group ($p<0.05$). Conversely, supplementation of SSE in preventive group increased TP compared to the positive control group ($p<0.05$). However, supplementation of SSE on therapeutic group showed no significant different when compared to positive control group ($p>0.05$).

Similarly, there were no significant difference observed when comparing the negative control group with the preventive control and therapeutic control group ($p>0.05$). On the other hand, the levels of CD were found to be significantly increased in all DEN-treated group ($p<0.05$) when compared to negative control (Fig. 2) regardless with or without SSE treatments. Interestingly, the administration of SSE in preventive group and therapeutic group reduced the levels of CD when compared to the negative and positive control group ($p<0.05$). It is noteworthy that pre-supplementation of SSE in 4 weeks shows more prominent protective effects on the liver compared to 12 weeks post-supplementation ($p<0.05$).

On the other hand, the levels of MDA were found to be significantly increased in positive control group compared to negative controls group ($p<0.05$) (Fig. 3). Concurrently, supplementation of SSE in both preventive

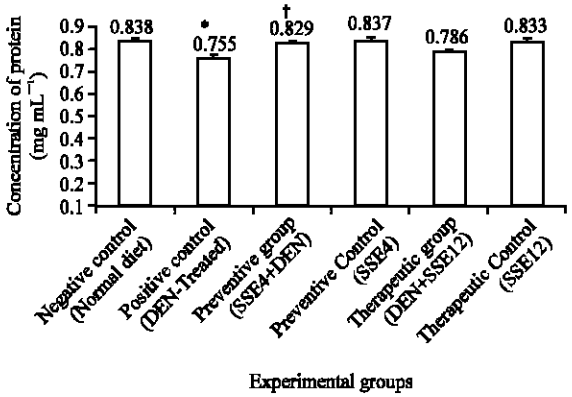

Fig. 1: Levels of Total Protein (TP) in the liver of all experimental groups. *p<0.05 significant as compared with negative control group; †p<0.05 significant as compared with positive control group

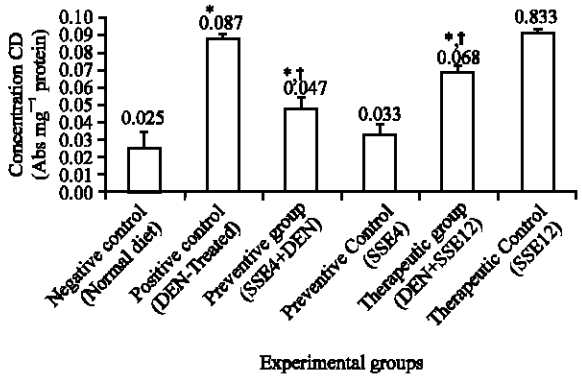

Fig. 2: Levels of Conjugate Dienes (CD) in the liver of all experimental groups. *p<0.05 significant as compared with negative control group; †p<0.05 significant as compared with positive control group

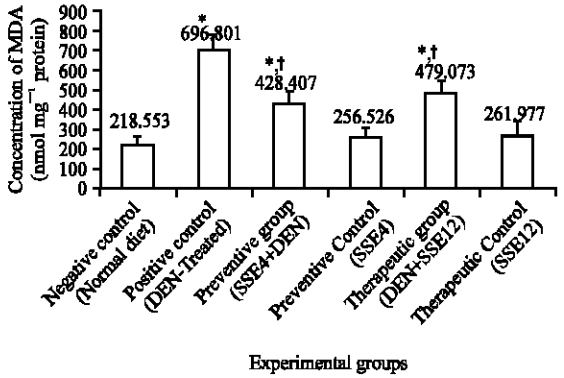

Fig. 3: Levels of malondealdehyde (MDA) in the liver of all experimental groups. *p<0.05 significant as compared with negative control group; †p<0.05 significant as compared with positive control group

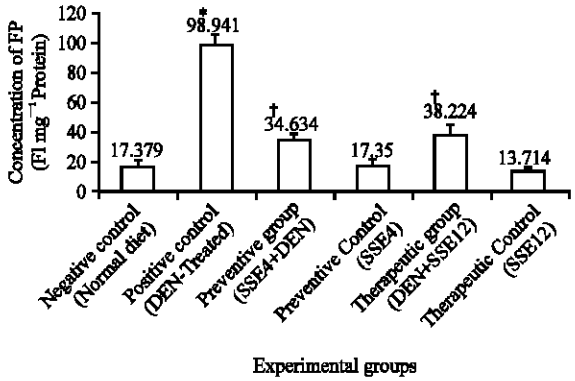

Fig. 4: Levels of Fluorescent Pigment (FP) in the liver of all experimental groups. *p<0.05 significant as compared with negative control group; †p<0.05 significant as compared with positive control group

and therapeutic groups showed marked decrease in the level of MDA when compared to positive and negative control groups (p<0.05). Nevertheless, treatment of SSE on the preventive and therapeutic control groups did not showed a significant different compared to the negative control group (p>0.05).

Similarly, a prominent increased of FP were observed in positive control group with as much as 470% compared to the negative control group (p<0.05). On the other hand, a significant decreased in FP concentration were seen when comparing the positive control group with the preventive and therapeutic groups (p<0.05) (Fig. 4). Even though there were no significant different observed among the negative control, preventive control and therapeutic control groups (p>0.05), it is worth to note that supplementation of SSE in both preventive and therapeutic groups able to drop the levels of FP to the extent of negative control (p>0.05).

As expected, the level of AOA was reduced in the positive control group compared to negative control group (p<0.05). However, supplementation of SSE in both preventive and therapeutic groups manage to bring up the level of AOA significantly (p<0.05). Contrary, there were no significance difference observed in the levels of AOA among the negative control, preventive control and therapeutic control groups of rats (p>0.05) (Fig. 5). Nonetheless there was a weak negative correlation observed between AOA and CD (r = -0.397, p<0.05) and also AOA and FP (-0.427, p<0.05).

In the assessment of DNA damage, comet assay showed a significant increased in the mean of tail moment in the positive control group compared to negative control group (p<0.05). There were no marked different observed among the negative control, preventive control and therapeutic control groups (p>0.05). Pre-treatment

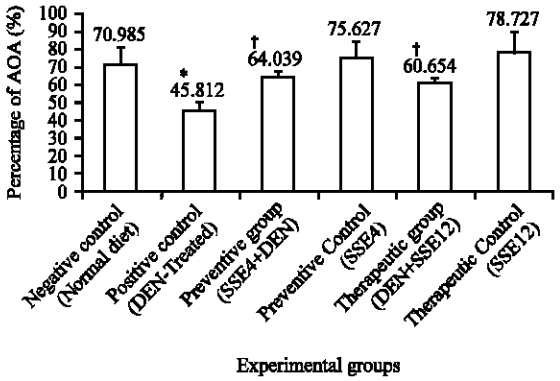

Fig. 5: Levels of antioxidant activity (AOA) in the liver of all experimental groups. *p<0.05 significant as compared with negative control group; †p<0.05 significant as compared with positive control group

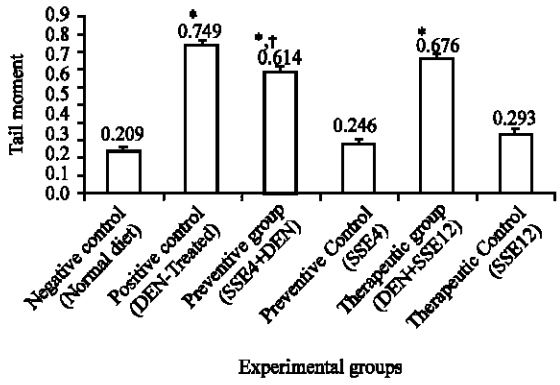

Fig. 6: Length of tail moment of the liver of all experimental groups. *p<0.05 significant as compared with negative control group; †p<0.05 significant as compared with positive control group

with SSE 4 weeks (preventive group) significantly reduced the mean of tail moment when compared to positive control group (p<0.05) (Fig. 6). Conversely, post-treatment of SSE 12 weeks (therapeutic group) do not showed a significant improvement when compared to the positive control group (p<0.05). Nevertheless, both preventive and therapeutic groups of rats showed an increment of mean tail moment than negative control group (p<0.05).

DISCUSSION

In this study, DEN-treated rats without supplementation of SSE significantly decreased TP compared with control group and this finding is in line with the findings of Thirunavukkarasu and Sakthisekaran (2003b) which also showed the same results. Similarly, increased catabolism which leads to hypoalbuminemia was also observed on Morris hepatoma (Rotermund *et al*., 1970). As reported previously, DEN may act as liver tumor inducer in rats (Jahan *et al*., 2011), therefore the decrease in albumin levels in DEN-treated rats might be due to the infiltration of tumor which leads to impaired hepatic function.

Oxidative stress is believed to act as major contributor in the pathogenesis and progression of liver diseases (Sulaiman *et al*., 2006). Several studies have shown that administration of DEN will produce free radical which will then cause increment of oxidative stress. Through this, DEN may initiate a series of mutagenic environment which lead to cancer (Thirunavukkarasu and Sakthisekaran, 2003b). The levels of CD, MDA and FP observed in this study were lower in SSE-treated rats pre- and post-hepatoma compared with DEN-tereated rats without supplementation of SSE. This findings supported by the previous studies from Thirunavukkarasu and Sakthisekaran (2003b) which showed that supplementation of selenium in the form of SSE can decrease nodular incidence and multiplicity in all three stages (before initiation, during initiation and promotion stages) in a significant manner. At the same time, toxic symptoms were not observed in SSE alone treated rats. These findings are in agreement with Thirunavukkarasu and Sakthisekaran (2003b). Selenium has been identified as important component of enzymatic active sites for GPx (Gallegos *et al*., 1997) and plays vital role in detoxifying hydrogen peroxide (Ozdemir, 2011). Selenium also inhibits growth of different cancer cells through the induction of apoptosis (Gallegos *et al*., 1997; Powis *et al*., 1997). Selenium supplementation was observed to decrease superoxide anion production by inhibiting formation of DNA adduct and minimizing membrane destruction (Ozdemir, 2011).

In this study, AOA was found to be higher in SSE treated rats pre- and post-hepatoma compared with DEN-treated rats without supplementation of SSE. Kuroda *et al*. (1985) reported that lower serum AOA was associated with higher MDA. This showed lower serum AOA was more vulnerable to cellular destruction induced by lipid peroxidation. Pigeolet *et al*. (1990) cited that antioxidants were used during increased lipid peroxidation on cancer tissues.

The intensity of DNA damages which indicated through length of comet tail observed in comet assay showed a significant different in the DEN-treated group compared to negative control group. Previous study has shown that DNA oxidation which induced by Reactive

Oxygen Species (ROS) is one of the major cause of DNA damages and may be mutagenic and lead to cancer. Act as a strong damaging agent, ROS may break the chain and cause changes in the guanine and tiamine base and also changes in the chromatin pairs. These changes will deactivate the tumor suppressor gene and increase the expression of proto-oncogene (Brown and Bicknell, 2001). On the other hand, the product of lipid and protein oxidation process will also contribute in the formation of DNA adduct which may cause mutation (Marnett, 2000). ROS generate various modified DNA bases. Among them 8-oxo-7,8-dihydroguanine (8oxoG) is the most abundant and seems to play a major role in mutagenesis and in carcinogenesis by playing a role in the process of initiation, promotion and progression of tumor (Cooke *et al*., 2003). Concurrently, the protection role of SSE on the DNA strand breaks has been grant from the previous researches and supplementation of Selenium has been proven to prevent DNA damages induced by DEN and Phenobarbital on the hepatoma cells (Thirunavukkarasu and Sakthisekaran, 2003b).

Conversely, rats with supplementation of SSE pre-hepatoma are less exposed to damage cause by oxidative stress compared to rats with supplementation of SSE post-hepatoma. This findings is supported by the study of Thirunavukkarasu and Sakthisekaran (2003a) which also reported that protective role of SSE was more prominent during hepatocarcinogenesis which is observed before the initiation phase of carcinogenesis compared with promotion phase. This phenomenon is due to the DEN metabolism was required for initiation of carcinogenesis. Terol *et al*. (1986) cited that lipid peroxidation can be inhibited during initiation phase of carcinogenesis by free radical scavenger and antioxidants.

CONCLUSION

Findings of this study indicated that the administration of SSE significantly revert the oxidative damages induced by DEN. It is noteworthy that supplementation of SSE in the normal healthy condition neither significantly add up the antioxidant properties nor raising the oxidative stress condition. However, the chemoprevention or protective effect of SSE was more pronounced when it was supplemented before initiation phase of cancer when compared to promotion phase.

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