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Harliansyah



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## Research Article

### VIABILITY AND APOPTOTIC EFFECT of SELENITE ON HUMAN LIVER CANCER CELL LINE (HepG2)

Harliansyah

Department of Biochemistry, Faculty of Medicine, University of YARSI  
Jakarta. 10510 Indonesia

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#### ABSTRACT

One of the attractive strategies considered in current cancer prevention or therapy is to induce death of malignant cells through apoptosis. Numerous studies in animal models and more recent studies in human have demonstrated cancer preventive effects with selenium. The aim of this study was to investigate effects of sodium selenite to induced apoptosis as well as to inhibit growth, proliferation in human hepatoblastoma (HepG2) cells compared in normal liver cell line (WRL-68). The results of this study showed that sodium selenite reduced significantly viabilities of WRL-68 and HepG2 cells with  $IC_{50}$  46.67 and 51.97  $\mu$ M respectively. Analysis of the DNA fragmentation with BrdU assay, showed that selenite also induced apoptotic in liver cancer cell line compared with control. The induced significantly apoptotic values by  $3.03 \pm 0.77$  %,  $4.67 \pm 0.79$  % and  $34.07 \pm 4.66$  % in HepG2 cell line at 25, 50 and 100  $\mu$ M respectively. However selenite had apoptotic values by  $10.77 \pm 0.007$  %,  $14.3 \pm 0.019$  % and  $20.37 \pm 0.007$  % in normal cell (WRL-68) at 25, 50 and 100  $\mu$ M respectively. This study showed that selenite had inhibitory effects on the viability and DNA synthesis of human hepatoblastoma cells (HepG2).

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#### INTRODUCTION

Hepatoma cellular carcinoma is one of the most common cancers in the world, with an annual incidence of approximately 1 million deaths, mainly in under developed and developing countries (Pang *et al.*, 2006). An imbalance between proliferation and apoptosis is strongly linked to the cause of most cancers including liver cancer (Farinati *et al.*, 2001). The search for cancer chemoprevention agents found in natural product or micronutrients is gaining a lot of interest in cancer research (Gossiau and Chen, 2004).

Selenium (Se) is a dietary essential micronutrient with important physiological functions in organism. Selenium can protect human body against oxidative damage involving in synthesis of glutathione peroxidase (GPx). It is known that free radicals are involved in the hypoxia or ischaemic stress and carcinogenesis and accompanying with the change of the activity of antioxidative enzyme in vivo. In the last two decades, selenium compounds have been extensively studied as chemopreventive agents against cancer (Rusolo *et al.* 2015; Bost and Blouin, 2009). It is uncertain whether Se influences tumor growth and leads to proliferation of hepatoma cells (Navsariwala and Diamond, 2004).

Epidemiological investigations indicate the inverse correlation of selenium intake level with cancer risk in human, and experimental studies with animal models also strongly support the chemopreventive effect of selenium compounds. Based on experimental studies, the anti carcinogenic activity of selenium mainly depends on two important factors, the dosage and the chemical form (Liu *et al.*, 2000).

The mechanisms of chemoprevention from selenite are strongly influenced by its metabolism. After absorption, selenite is reduced by glutathione (GSH) and NADPH dependent reductase through selenodiglutathione (GSSESG) to the highly toxic  $H_2Se$ , which can be converted to selenophosphate and then incorporated as, selenocysteine into selenoproteins, such as glutathione peroxidase, type-1 iodothyronine deiodinase, thioredoxin reductase and selenoprotein P. Selenium-containing enzymes are not as important as selenium metabolites in cancer prevention at levels above nutritional requirements. However, there is strong evidence to support the role of  $H_2Se$ , which is methylated to mono, di and trimethylated derivatives before excretion (El-Bayoumy and Sinha, 2004).

\*Corresponding author: Harliansyah

Department of Biochemistry, Faculty of Medicine, University of YARSI Jakarta. 10510 Indonesia

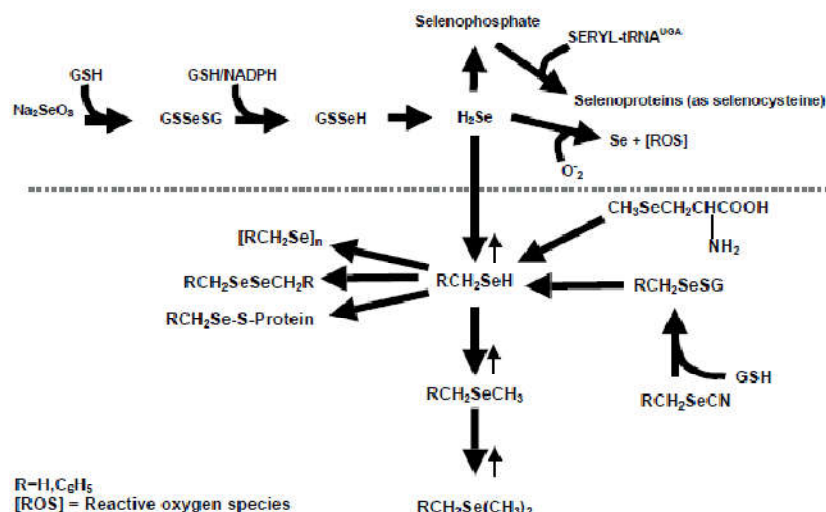


Fig. 1 Metabolic pathways of inorganic selenite and organoselenium compounds.

## MATERIAL AND METHODS

A series of cell lines (ATCC, Rockville, MD) were investigated in this study including; HepG2 cells (HB-8065) and a human fetal hepatocyte cell line, WRL-68 (C1-48). They were grown in 75 cm<sup>2</sup> tissue culture flasks in *Eagle Minimum Essential Medium* (EMEM Flow Lab, Australia, Cat no: 10-101-26) and supplemented with 10% fetal calf serum at 37<sup>0</sup> C, 5% CO<sub>2</sub>. HepG2 cell lines was selected for this study due to its consistent growth and ease or maintenance. Cellular viability was determined using MTS assays (Promega Kit). In brief, exponentially growing cells were harvested and 100µl of cell suspension containing 2x10<sup>4</sup> cells was in 96–well micro titer plates. After 24h of incubation to allow for cell attachment, the cell were treated with varying concentrations of selenite in medium (100µl) and incubated for 24h at 37<sup>0</sup> C under 5% CO<sub>2</sub>. Three hours after the addition of MTS solution, the amount of formazon formed was measured spectrophotometrically at 490 nm with plate reader. Apoptotic assay was determined using BrdU –DNA fragmentation (Roche Kit). Determination nuclear morphology of different cell types was using by fluorescence method employing propidium iodide.

## RESULTS

Cell viability can be defined as the number of healthy cells in sample. Viability is measured by the ability of cells with uncompromised membrane integrity to exclude the dye. Analysis by MTS assay shown reduced of viability in liver cancer cell line (HepG2). The results of this study showed that sodium selenite reduced significantly viabilities of WRL-68 and HepG2 cells with IC<sub>50</sub> 46.67 and 51.97 µM respectively (Figure-2).

Figure 3 showed that sodium selenite can induction of apoptosis in human hepatoblastoma (HepG2) cell. The induced significantly apoptotic values by 3.03 ± 0.77 %, 4.67 ± 0.79 % and 34.07 ± 4.66 % in HepG2 cell line at 25, 50 and 100 µM respectively. However selenite had apoptotic values by 10.77 ± 0.007 %, 14.3 ± 0.019 % and 20.37 ± 0.007 % in normal cell line (WRL-68) at 25, 50 and 100 µM respectively.

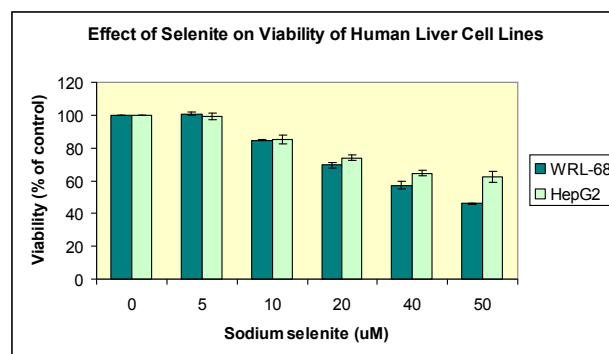


Fig.2 Viabilities of HepG2 and WRL-68 cells with treatment of selenite.

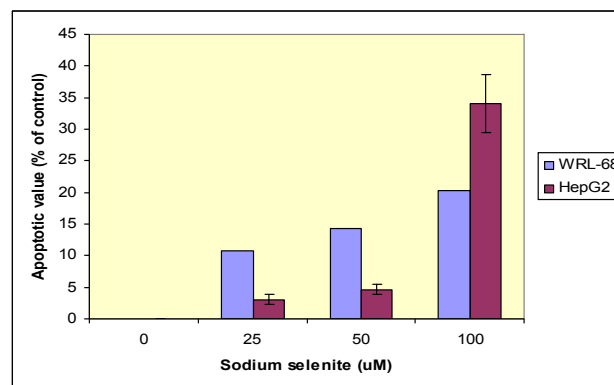
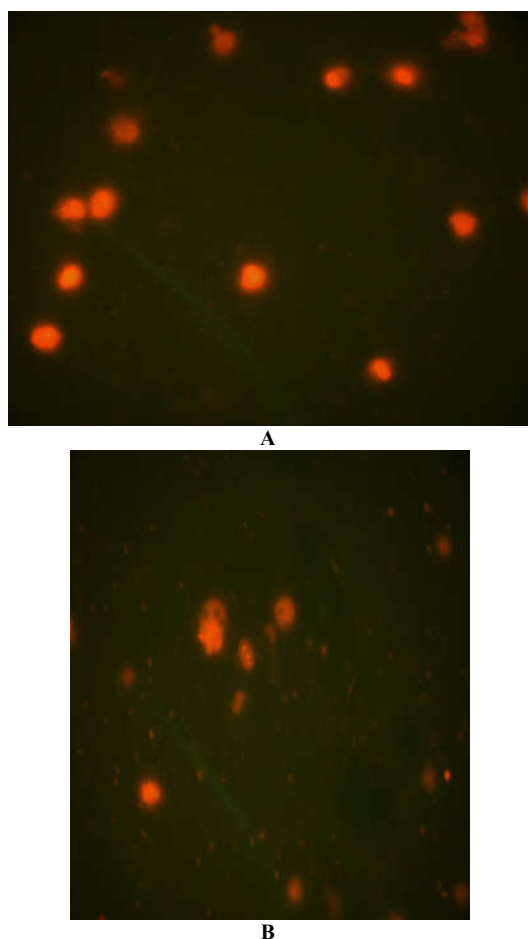


Fig.3 Apoptotic value of HepG2 and WRL-68 cells with treatment of selenite

In figure 4 showed that the use of a fluorescence method employing propidium iodide to determine nuclear morphology of liver cancer cell line (HepG2) and normal cell (WRL-68) by fluorescence microscopically.



**Fig. 4.A** HepG2 cell untreated of selenite and Fig 3B treated with 100  $\mu$ M. The apoptotic cells at 48 hours after treatment with 100  $\mu$ M selenite. Apoptotic features with nuclei condensation and fragmentation were seen as yellowish bodies, by using propidium iodide fluorescence photomicrograph.

## DISCUSSION

Measurements of cell viability, proliferation and cell death in the liver, are important in development, normal cell turnover, wound healing and tumor progression. The quantification of cell viability is an important parameter for the description of the status of cell cultures and is basis for numerous cytotoxicity studies. Cell viability can be reflected by the integrity of the mitochondria, when MTS reagent (a tetrazolium salt) is applied to living cells, it is converted to a color compound (formazon) with the emission of light at 490 nm.

The mechanism of chemoprevention based on induction of apoptosis, separate from toxic effects and independent of a functional p53, strengthens the case for Se chemoprevention in the human population. Since Se induced apoptosis can be dissociated from toxic effects other mechanisms must be involved. Se induced alterations in cell cycle proteins associated with G1/S phase and decreased DNA synthesis.

Apoptosis is the biological process by which cells in tissues undergo programmed death. It is a regulated form of cell suicide that is characterized by progressive cell shrinkage, nuclear condensation, cleavage of DNA into nucleosome sized fragments, loss membrane integrity, membrane blebbing and finally engulfment of intact cell fragments (apoptotic bodies). Elucidating the molecular basis about the apoptotic sensitivity of tumor cells will be important for understanding the process of tumor progression and also for applying the basic knowledge of tumor to clinical and therapeutic fields.

Understanding the metabolism of organoselenium compounds is essential to determine whether the parent compounds and / or its metabolites are responsible for chemoprevention (Venardos *et al*, 2004., El-Bayoumy, K., Sinha, R. 2005). In conclusion, selenite treatment was demonstrated to suppress tumor growth *in vitro* thus found to have an enhanced anti tumor effect on hepatoma cancer cells.

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