



POTENCY OF BRASSICACEAE EXTRACTS AS AN APOPTOSIS ENHANCER IN BLOOD CELLS MICE EXPOSED TO LEAD ACETATE

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ABSTRACT

Lead (Pb) is an environmental pollutant that is widely used in various industries. It has genotoxic effect that can trigger cancer formation (carcinogenic). The member of Brassicaceae family (white headed cabbage, pak choi, and Chinese cabbage) is known to have glucosinolates, isothiocyanates, phenethylisothiocyanates, and allyl isothiocyanates which are anticarcinogenic. The aim of this study was to examine the potential of Brassicaceae extracts to enhance apoptosis in mouse blood cell exposed to lead acetate. The research method was descriptive with five treatments and four replications. Mice were pretreated with white headed cabbage, pak choi, and Chinese cabbage extracts by cumulative dose 156 mg/kg bw for 13 days prior to exposure of lead acetate by cumulative dose 150 mg/kg bw. All treatment was given orally. Blood samples were taken on the thirteenth day from the tail venous vessel. Furthermore, the DNA isolation and electrophoresis were done to examine DNA fragmentation. The result showed that the white headed cabbage extract enhanced the formation of a 200 bp DNA ladder pattern that is characteristic of apoptosis in the mice blood cells due to lead acetate exposures.

Key words: apoptosis, lead acetate, white headed cabbage, pak choi, Chinese cabbage

INTRODUCTION

Heavy metals like Pb, Cd, Cu, Fe, and Hg, contribute significantly to the growing pollution problem. Lead (Pb) is used in the manufacture of batteries, metal products, paints and ceramic glazes; the large source of lead in the atmosphere has been from combustion. Lead is absorbed mainly by ingestion and inhalation. Internal and external factors, including environmental pollutants induced various kind of genetic damage. Lead is genotoxic itself or enhances the effect of other DNA-damaging agents that can trigger cancer formation.

Prevention of the cancer and other mutation related disease can be carry on both by avoiding exposure to carcinogen and by favoring the intake of protective factors which fortify physiological defense mechanisms (Ishaq *et al.*, 2003). In recent years, there is increasing awareness that certain naturally occurring substance in plants provide protection against environmental carcinogen or mutagens. The *Brassicaceae* (*Cruciferae*) family has chemopreventive potential (anti carcinogenic). Anticarcinogenic effect of these vegetables, particularly due to their content in glucosinolate, is hydrolyzed by specific thioglucosidases called myrosinases to produces isothiocyanates, nitriles, and thiocyanates with different biological activity (Rubattzy & Yamaguchi, 1994; Talalay & Fahey, 2001; Miyazawa *et al.*, 2003; Weil *et al.* 2004; Mandelova & Totusek, 2007). Isothyosianate (ITC) may also be able to suppress tumor cell growth, and it has been recognized that isothiocyanates can inhibit cell cycle progression and trigger apoptosis in tumor cells (Tseng *et al.*, 2004). The most characterized ITC is sulforaphane, it has ability to simultaneously modulate multiple cellular target involved in cancer development, including: (1) DNA protection by modulating metabolizing

enzyme and blocking the action of mutagens; (2) inhibition of cell proliferation and induction of apoptosis; (3) inhibition of neoangiogenesis (Fimognari & Hrelia, 2007). This study was conducted to examine the anticarcinogenic potential of *Brassicaceae* vegetables (*Brassica rapa* L. cv. Group Pak Choi, *Brassica Oleracea* L. cv. Group White Headed Cabbage, and *Brassica rapa* L. cv. Group Chinese Cabbage) extracts to enhance apoptosis in mice blood cell that exposed to lead acetate.

MATERIALS AND METHODS

Animal

Animal experiments were 8-10 week old Swiss Webster male mice weighing 20-25 g obtained from Structure and Developmental Animal Laboratory, Faculty of Mathematics and Natural Sciences, Padjadjaran University. The animals were maintained at $\pm 25^{\circ}$ C with 12 hr light and dark cycle. Animal were fed with commercial standard mouse diet (CP 551) and water ad libitum.

Chemicals and substances

Lead Acetate (Merck), Carboxy Methyl Cellulose (Merck) as emulsifier, 95% diethyl ether, extracts from *Brassicaceae* vegetables (*Brassica rapa* L. cv. Group Pak Choi, *Brassica oleracea* L. cv. Group White Headed Cabbage, and *Brassica rapa* L. cv. Group Chinese Cabbage), Apoptotic DNA Ladder Kit (Roche; Cat. No. 11 835 246 001.) and 100 bp-DNA marker (Roche; Cat. No. 11 449 460 001*).

Extraction

The *Brassicaceae* vegetables were cut and blended. Ethanol extract was prepared by extraction of leaves in 95% ethanol in a soxhalet apparatus for 72 h. The liquid extract was then cooled and concentrated by rotary evaporation at 40°C to get pasta form-extract.

Treatment Procedure

Twenty of 8-10 weeks old mice weighing 20-25 g were allowed without food for 4 h before treatment. There were five experimental groups and each group consisted of four male mice. Group I of negative control was administered with CMC 0.5% (KN), group II of positive control was administered with 150 mg/kg bw lead acetate (KP) (Aly, 2001). Groups III, IV, and V were pretreated with 156 mg/kg bw of pak choi extract (PK), 156 mg/kg bw white headed cabbage extract (KC) and 156 mg/kg bw Chinese cabbage extract (PT) respectively for 7 (1, 3, 5, 7, 9, 11, and 13) consecutive days (22.22 mg/kg bw/day) prior to treatment with lead acetate dose of 150 mg/kg bw on day 5, 9 and 13 (50 mg/kg bw/day). All treatment was given orally.

DNA Isolation and Electrophoresis

Formation of DNA fragments in apoptotic cell death in each treatment group was detected from the blood sample. An hour after last treatment (day 13) , blood sample was taken from the tail and DNA was isolate by the method of Roche using Apoptotic DNA Ladder Kit. As an apoptotic positive control was U937 cell (Roche). DNA concentration was measured

by biophotometer at wavelength of 230, 260 and 280 nm. DNA was then electrophoreses on a 1% agarose gel using 100 bp DNA marker (Roche). Electrophoresis was using 2 ug of DNA sample and carried out at 75 V for 1.5 hours. Gel was stain by ethidium bromide solution for 10 min and distilled water for 5 min. DNA bands were visualized and documented by UV transilluminator. Formation of a 200 bp DNA ladder pattern was indicating the occurrence of apoptosis in the mouse blood cell. The data were descriptively analyzed.

RESULT AND DISCUSSION

Electrophoregram of mice blood cell DNA from all treatments was shown in Figure 1. The results of DNA electrophoresis that isolated from the blood of mice showed that the treatment of lead acetate dose of 150 mg/kg bw caused DNA fragmentation (column 1, Figure 1). By using a same amount of DNA sample, thicker band was shown from sample was treated with white headed cabbage extract. This result indicated that white headed cabbage may enhanced apoptosis (column 14, Figure 1). However, the mechanism of apoptosis cell death induced by lead and isothiocyanates compounds contained in *Brassicaceae* vegetables occurs through different signal transduction pathways.

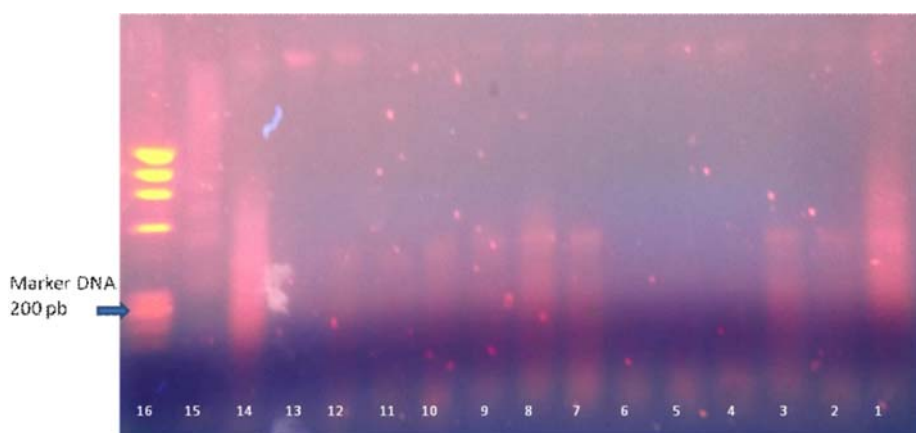


Figure 1. Electrophoregram that showing DNA ladder pattern from apoptotic mice blood cells.

| | | | | |
|-------|--------|--------|---------|----------------|
| Ket.: | 1. KP1 | 5. KP3 | 9. PT1 | 13. KC2 |
| | 2. KP2 | 6. PK1 | 10. PT2 | 14. KC3 |
| | 3. KN1 | 7. PK2 | 11. PT3 | 15. sel U937 |
| | 4. KN2 | 8. PK3 | 12. KC1 | 16. DNA Marker |

The regulations of apoptosis are complex and two important pathways are involved. Apoptosis is regulated by Bcl-2 protein family that consists of anti-apoptotic proteins such as Bcl-2, Bcl-xl, Bcl-w, and pro-apoptotic proteins such as Bax, Bak, and Bad. In extrinsic apoptosis, external signals interact with death receptors, which in turn activate caspase-8 subsequent to the activation of caspase-7 and caspase-3 as an effectors process of apoptosis, such as caused by isothiocyanate compounds. In intrinsic apoptosis, the loss of mitochondrial transmembrane potential caused by accumulation of Ca^{2+} ion, and together with Bax protein may initiate cytochrome *c* release, which binds to apoptotic activating factor-1 (Apaf-1). Oligomer of Apaf-1 then binds to procaspase-9 form apoptosom (complex activation of Caspase-9). Caspase-9 subsequently activates caspase-3 as an effector apoptotic process, such that caused by lead. In multiple cell types, activated caspase-3 will

result in certain apoptotic hallmarks, such as chromatin condensation and DNA fragmentation (Xu *et al.*, 2006).

Lead toxicity is thought to relate to its affinity for some protein and can mimic Ca^{2+} ion action. Lead interact with enzyme functional groups and high-affinity metal-binding proteins, such as Pb-binding proteins and metallothioneins, can mediate this Pb-enzyme interaction. Lead exposure can induce DNA damage that leads to increased expression of protein Bax and decreased the expression of Bcl-2 protein. DNA damage activates p53 which in turn triggers Bax gene expression resulting in an imbalance ratio between Bax to Bcl-2 and interfere with mitochondrial function. This will activate caspase-3 and causes the cell to undergo apoptosis (Xu *et al.*, 2006).

According Barillari *et al.* (2007), vegetables of the *Brassicaceae* family contain glucosinolates compound which can hydrolyze into isothiocyanates in the digestive tract. Isothiocyanates compound contained in *Brassicaceae* vegetables are function as anti oxidants, anti cancer and anti mutagen, that can induce apoptosis (Nakamura *et al.*, 2001; Barillari *et al.*, 2007). Isothiocyanates can be converted into glutathione conjugates with the aid of the enzyme glutathione S-transferase (GSTs). Glutathione conjugate, S-(N-Phenethylthiocarbamoil) glutathione, will be taken out of the cell and metabolized into mercapturic acids, N \acute{a} -acetyl-S-(N'-Phenethylthiocarbamoil) cysteine . This compound was able to induce apoptosis in cancer cells and inhibit cancer growth by activating enzymes that play a role in the detoxification process such as quinone reductase and epoxide hydrolase (Xu & Thornalley, 2001).

One class of isothiocyanate compounds is sulforaphane. The in vivo and in vitro study results suggest that sulforaphane can modulate early stages of cancer formation (initiation stage), such as apoptosis, cell proliferation, and angiogenesis (Fimognari & Hrelia, 2006). Inhibition of genetic damage by isothiocyanate compounds or suspected sulforaphane was an early mechanism to prevent the onset of cancer. Inhibitory mechanism occurs through the inhibition or competition with enzymes that metabolize xenobiotic (phase I and II detoxification enzymes), scavenge free radicals, and protect the nucleophilic sites on DNA, namely through the formation of complex with mutagens or modulate cell repair, cell cycle, or cell death via apoptosis.

Thus, pretreatment with *Brassicaceae* extract dose of 156 mg/kg bw prior to lead acetate exposure dose of 150 mg/kg bw can enhance the occurrence of apoptosis in the mice blood cell, mainly the White headed cabbage. The mechanism of apoptosis occurs through the binding of mutagens, in this case lead ions, and formed a complex between lead ion-isothiocyanate compounds which can inhibit DNA damage, and prevent formation of cancer.

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