

PROTECTIVE EFFECT ETHANOLIC EXTRACT OF *Boesenbergia pandurata* (ROXB.) Schlecht. AGAINST UVB-INDUCED DNA DAMAGES IN BALB/C MICE.

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ABSTRACT

Boesenbergia pandurata (Roxb.) Schlecht. contains bioactive compounds that have a number healthy effect including anti-oxidant and anti-carcinogenic activities. This research was carried out to examine the protective effect of *B. pandurata* extract against expression of cyclobutane pyrimidine dimers (CPDs) as marker of UVB-induced DNA damage in Balb/c mice. Dried powder of *B. pandurata* rhizomes was extracted by maceration method using 90% ethanol. The extract was quantified with pinostrobin as active marker using TLC scanner. Ethanolic extract of *B. pandurata* (EEBP) was given orally at 14 days prior UV exposure with variety doses, 0 (vehicle), 20, 40 and 60mg/kgBW/day and continuing until termination of the experiment. Following the UVB irradiation (1.4J/m²), mice were sacrificed at different time points (2, 24, 48, and 72h after UVB exposure). The back skin samples were collected to analyze CPDs expression by immunohistochemical method. The result showed that EEBP (contained 0.5% pinostrobin) dose was 40 and 60mg/kgBW/day had protective activity against UV-induced DNA damage as indicated by the decrease of CPDs expression.

Key words: *Boesenbergia pandurata* (Roxb.) Schlecht., UVB, DNA damage, CPDs.

INTRODUCTION

The solar ultra violet (UV) radiation is main factor caused skin cancer (Katiyar, 2007). Overexposure solar UV radiation induce oxidative stress, inflammation, immunosuppression, and DNA damage, which has been implicated in variety of skin damages including erythema, sunburn, scaling, dryness, premature aging, and skin cancer (Katiyar, 2007; Pérez-sánchez *et al.*, 2014). UVB radiation can be absorbed by chromophores of DNA resulting to the formation of DNA lesion primarily *cyclobutane pyrimidine dimers* (CPDs) and (6-4)-pyrimidine pyrimidones photoproduct (Matsumura and Ananthaswamy, 2004). These photoproducts are UVB-induced DNA lesions specific. Between various types of DNA damage, CPDs is most plentiful, difficult repaired, and have mutagenic activity (You and Szabo, 2000).

The development of chemopreventive agents and strategies on reducing the risk of UV-induced skin cancer was required to repress this health issue (Katiyar, 2007). Naturally

product compounds which possess antioxidant, anti-inflammatory, immunomodulatory, and increase DNA repair system can be exploited as ideal chemopreventive agents for skin cancer (Vaid and Katiyar, 2010). One of the natural products is come from *Boesenbergia pandurata*, (Roxb.) Schlecht., known as *temu kunci* in Indonesia, *fingerroot* (English) and *kra-chai* (Thailand). The rhizome has been used for a spice and folk medicine such as treatment of asthma and gastrointestinal disorder (Shindo *et al.*, 2006; Tanjung *et al.*, 2013).

Studies on the rhizomes extract of *B. pandurata* have showed cytotoxic activity (Kamkaen *et al.* 2006) and anti-proliferatif effect against ovarian (CaOV₃), breast (MDA-MB-231 and MCF-7), cervical (HeLa) and colon (HT-29) cancer cell-lines (Jing *et al.*, 2010). The rhizomes of *B. pandurata* contain bioactive compound flavonones (pinostrobin, pinocembrin, alpinetin) and chalcones (cardamomin, boesenbergin-A, panduratin-C, panduratin-A, hydroxypanduratin-A)

(Cheenpracha *et al.*, 2006; Ching *et al.*, 2007). Boesenbergin-A has been shown to apoptosis induce against human non-small cell lung cancer (A549 cells) (Isa *et al.*, 2012). Panduratin A, demonstrated anti-proliferative effect and induced apoptosis on human breast cancer (MCF-7) and human colon adenocarcinoma (HT-29) cell-line (Kirana, *et al.*, 2006), this compound also could inhibit atopic dermatitis (Kim, *et al.*, 2014). While both pinocembrin and pinostrobin showed cytotoxic effect in breast cancer (T47D) and colon cancer (WiDr) cell-line (Tanjung *et al.*, 2013). All the activity of these substances isolated from *B. pandurata* are correlated with chemoprevention action.

This research was carried out to examine the protective effect of EEBP against of expression cyclobutane pyrimidine dimers (CPDs) that was specific lesion of UV-induced DNA damage in Balb/c mice

MATERIALS AND METHODS

Plant materials

Rhizomes of *B. pandurata* were collected from Kalibawang, Kulonprogo, Yogyakarta, Indonesia, and determined by a taxonomist from Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada. These rhizomes was sliced and dried at 50°C then grounded to a fine powder, followed by maceration process using 90% ethanol for 72h, with occasional stirring. This process was repeated for three times and all the macerate obtained then evaporate using vacuum rotary evaporator to get semi solid ethanolic extract (Nihlati *et al.* 2008). The extract was then analyzed pinostrobin contained using TLC (Thin Layer Chromatography) and TLC scanner (Gamac TLC Scanner-3) for quantification. Isolated pinostrobin (HPLC purity of 96%) was obtained from Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta,

Experimental animals

Female 21 days old Balb/c mice were obtained from Integrated Research and Testing Laboratory, Universitas Gadjah Mada. These mice were fed *ad libitum* pellets (BR11) and drinking water. The experiment protocols were approved by the Ethical Clearance Committee of Integrated Research and Testing Laboratory,

Universitas Gadjah Mada (OR/KEC-LPPT/1/2011).

The mice were divided into 5 groups, control (drinking water), vehicle (solvent of EEBP + UVB), and three groups (EEBP with 20; 40; and 60mg/kgBW/day respectively + UVB). EEBP was given orally at 14 days prior UV exposure and continuing until termination of the experiment.

UV light radiation

The source of UV light is UVB lamps 270-290 nm wavelength (Dermatology, Medicine Faculty, Universitas Gadjah Mada). Mice were exposed to acute UVB irradiation 1.4 J/m².

Skin samples collection

At each time point (2, 24, 48, and 72h after UV exposure), three mice from each group were sacrificed, dorsal skin of mice were necropsied. The skin samples (3x3cm²) were fixed with 10% buffered formalin and embedded in paraffin, this process referenced from standard procedure of Histology and Cytology Laboratory, Faculty of Medicine, Universitas Gadjah Mada.

Detection of CPDs

Tissue section (5µm) were cut and mounted onto poly-l-lysine coated slides and endogenous peroxidase was quenched by 0.3% hydrogen peroxide. The sections were then denaturated in 70mMol/L NaOH in 70% ethanol for 2min and neutralization for 1min in 100mMol/L Tris-HCl (pH 7.5) in 70% ethanol. Non-specific binding sites were blocked with PBS containing 10% goat serum, followed by overnight incubating antibodies CPDs (CosmoBio). The CPDs immuno-reactivity was detected with LSAB2 System-HRP staining kit (Dako) and counterstained with Mayer's Hematoxylin.

Analysis of CPDs

Immunohistochemistry (IHC)-stained tissue were analyzed using a semi-quantitative system by image analyzer (ImageJ software). CPDs expression was interpreted in percent positive area of IHC stained. Statistic analysis was perform by ANOVA and followed by Tukey's test (SPSS 17.0).

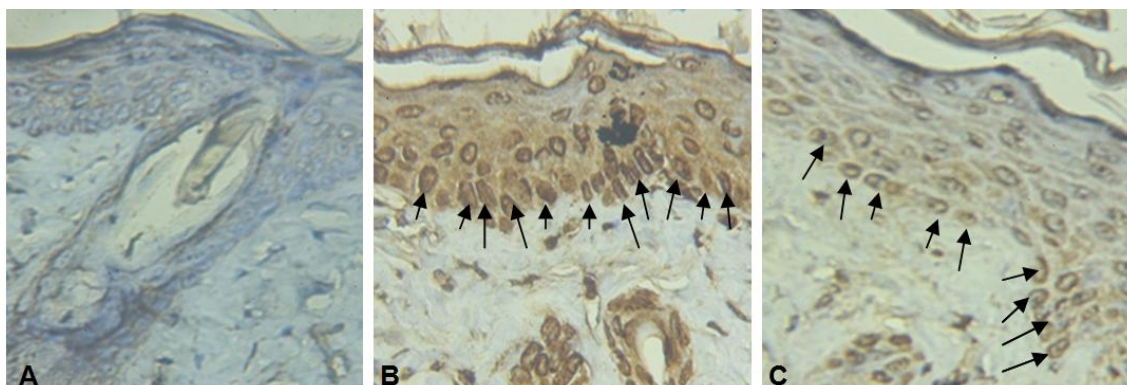


Figure 1. The observation of expression of CPDs using immunohistochemical method (original magnification 400x).

CPDs undetected on non UVB irradiation-group (A). Positively CPDs cells showed dark brown (→) found at dermal and epidermal layers in UVB treated groups only (B and C). Treatment of EEBP could decrease CPDs expression (C). (A): control group: without UVB and EEBP, (B) vehicle: solvent of EEBP+ UVB, (C) UVB + 60mg EEBP/kg BW/day

RESULT AND DISCUSSION

Rhizome of *B. pandurata* was contained some bioactive compounds, which showed antioxidant, anti-inflammatory, and anti-cancer activities potentially used photoprotective agent (Kirana *et al.*, 2007; Isa *et al.*, 2012). The different condition and area on collecting of the rhizomes, may affect the variation of compounds and activity. In this study, extraction of the *B. pandurata* rhizome produced a dark brown semi-solid extract, bitter and aromatic smell. The ethanolic extract of *Boesenbergia pandurata* (EEBP) contained 0.5% pinostrobin. The extract was given at 14 days before UVB exposure to examined chemopreventive effect against CPDs expression.

The acute exposure of UVB (1,4J/m²) on mice skin could induce DNA damage leading to the formation of CPDs. The dark brown color showed positively CPDs stained-cell (Figure 1). The CPDs expression was found in cytoplasm and nucleus cells of epidermal and dermal layer in UVB-exposed group (Figure 1). It was showed that UVB exposure penetrated into dermal layer skin mice, this result consistent with the previous study when UVB (180mJ/cm²) exposed on the SKH-1 hairless, C3H/HeN and IL-12p40KO mice (Meeran *et al.*, 2009; Afag *et al.*, 2010). UV-B radiation can penetrate the skin to depth approximate 160-180 μm. This rays can cross whole

epidermal layer and penetrate to dermal compartment of human skin (Nichols & Katiyar, 2010). Highest expression of CPDs showed at 2 hours after UVB exposure (Figure 2). Balb/c mice showed less sensitive to UVB irradiation than hairless mice which had the highest CPDs expression at 0.5h after 180mJ/cm² UVB irradiation on female C3H/HeN and IL-12p40 KO mice (Meeran *et al.*, 2009) and at 1h after a single dose of 3xMED (Minimal Erythema Dose) of Solar Simulator UV in hairless mice (Widyarini, 2006). These indicated that UVB-induced CPDs formation was influenced of wave length, intensity of radiation and animal sensitivity.

After 2h UVB irradiation, on EEBP-fed mice showed CPDs expression fewer than vehicle group (solvent of EEBP + UVB) (Figure 2), it demonstrated EEBP could reduce and remove CPDs⁺ cells rapidly in the skin of Balb/c mice. On vehicle group, positive CPDs stained-cells (CPDs⁺ cells) reduced (15%) within 24h after UVB exposure, and decline continued following time point of observation. These CPDs⁺ cells persisted up to 72h post-UVB. This finding supported the previously reported studies on UV induced-hairless mice (Zattra *et al.*, 2009). CPDs expression in group with 20 mg/kgBW/day EEBP treatment were not significantly

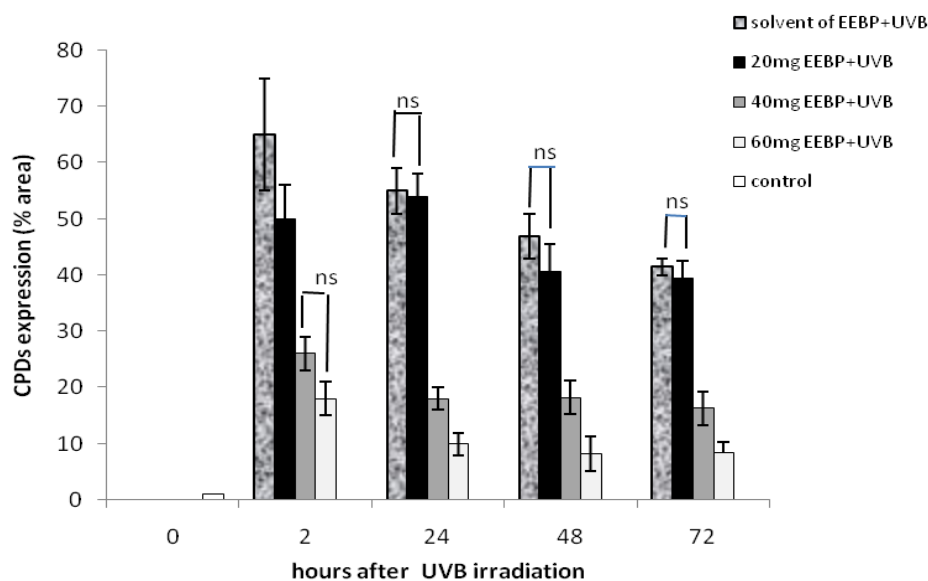


Figure 2. The representation of semi-quantitative analyze of effect of EEBP against CPDs expression.

CPDs positive (CPDs+) cells are expressed as percent area CPDs+. After 2 hours UVB irradiation, CPDs expression on EEBP-fed mice showed fewer area than vehicle group (solvent of EEBP +UVB), EEBP dose was 40 and 60 mg/kg BW/day decreased CPDs expression until 78% and 82% at 72 hours post-UVB. CPDs were undetectable (0%) on control group at all time point observation. (ns: not significant at the same time point; $p < 0.05$).

different with vehicle ($P < 0.05$) at 24, 48, and 72h after UVB irradiation, while groups with 40 and 60 mg/kg BW/day EEBP treatment could decrease of CPDs expression 60% and 72% from the vehicle value at 24h after UVB irradiation, 78% and 82% respectively at 72h after UVB irradiation. The EEBP activity however, has slightly less activity than isoflavone equol, since this compound was able to reduce $\pm 47\%$ of the CPDs expression at 24 hours and complete disappeared at 168h after UVB irradiation (Widyarini, 2006). The decrease of CPDs expression seemed to be caused by DNA repair activity through nucleotide excision repair. When DNA repair was limited, DNA damage were retained in the genome, therefore caused mutations and tumor formation (Marrot & Meunier, 2008). Therefore, both EEBP dose (40 and 60 mg/kgBW/day) showed protective effect against UVB induced DNA damage. This result were in accordance with previous studies, which indicated that natural diet agents including fruits, vegetables, and spices had ability to repress UV-induced skin cancer

through remove or decrease the DNA damage. Acceleration of UV-induced CPDs removal were demonstrated by other natural compounds from extracts of *Polypodium leucotomos* (Zattra *et al.*, 2009), *Punica granatum* fruits (Afag *et al.*, 2010), green tea polyphenol (Katiyar *et al.*, 2007; Roche *et al.*, 2010), and luteolin (Wolfe *et al.*, 2011). The mechanism of decreasing CPDs might be through antioxidant activity, increasing IL-12 (cytokine interfere formation of CPDs), p53 and p21 protein (Katiyar, 2007; Zattra *et al.*, 2009; Afag *et al.*, 2010; Wolfe *et al.*, 2011). *B. pandurata* rhizome has been reported contain essential oil, pinostrobin, alpinetin, pinocembrin, cardamonin, boesenbergin, panduratin, and others (Ching *et al.*, 2007), they might be contribute and affect on signaling pathway of DNA repair activity and therefore could decrease, inhibit and remove formation of CPDs. Pinostrobin, a flavonoid from *B. pandurata* has been reported to demonstrate antioxidant activity (Shindo, *et al.*, 2006; Nihlati *et al.*, 2008). It might be contribute to prevent CPDs formation was like mechanism of

flavonoid lutein, through free radical scavenging activity.

The previous studies demonstrated that orally treatment of EEBP exerts anti-wrinkling, moisturizing effects, and prevent of tumorigenesis (Kim, *et al.*, 2012; Listyawati *et al.*, 2012). In addition the extract was reported safe for consumption as *in vivo* studies showed no significant changes in the body weight of EEBP fed rats. Furthermore, all haematological and histopathological parameters used to evaluate the toxicity effect did not show any adverse changes (Saraihong *et al.*, 2012). Those results were suggest that EEBP showed potential as an effective systemic photoprotective agent for prevention of UVB-induced skin damages.

CONCLUSION

The result of study indicated that oral administration of EEBP 40 and 60 mg/kgBW/day could protect UVB-induced DNA damages through reduce CPDs formation. The extract could be more studied to clarify a functional agent which has photoprotective effect. Clinical studies are also required to characterize the beneficial effects of dietary EEBP on the photodamage of human skin.

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