***Original article:***

# Protective Effect of Bixin Isolated from *Bixa orellana* L. Seeds on UVB-Induced Inflammation and Immunosuppression of the Skin

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**Abstract**

***Background****:* DNA damage caused by excessive ultraviolet B (UVB) radiation on the skin triggers the response to inflammatory and immunosuppression. The bixin from *Bixa orellana* L. has been proven to be able to inhibit cyclo-oxygenase. ***Objective:*** to verify whether the bixin lotion has the effect to offer protection against inflammation and immunosuppression due to acute UVB irradiation in shaved BALB /c mice. ***Methods****:* Protection against inflammation and immunosuppression, respectively were studied in 4 groups of mice. Each group was treated respectively with topical application of base lotion as a control; bixin lotion doses of 0.5 mg; 2.5 mg and 125 mg, for 10 days prior to and during the UVB irradiation. The Inflammation was induced by UVB irradiation, 360 mJ/cm2 once a day for 3 consecutive days, whereas the immunosuppression was induced by UVB irradiation, 360 mJ/cm2 once a day for 5 consecutive days. The inflammatory response was measured as an increase in middorsal skinfold thickness at the peak response. The immune response was measured as the contact hypersensitivity (CHS) response to oxasolon sensitization. ***Results****:* The results indicated that in concentration range used, bixin lotion significantly decreased the middorsal skinfold thickness at 72 hours after UVB radiation ($p < 0.05$) compared to the control, but there was no significant difference between couples of the dose of bixin. Bixin lotion was also capable to restore the suppression of CHS from 34.22% in the control group to 11.4%; 0.5% and 0% at doses of 0.5; 2.5 and 125 mg respectively ($p < 0.05$). ***Conclusion:*** Bixin lotion has the potential to reduce the inflammatory edema reaction and the suppression of CHS of mice induced by UVB radiation.

**Keywords**: *Bixa orellana* L.; photoprotection; inflammation; immunosuppression; UVB radiation

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## Introduction:

Ultraviolet radiation containing UVB is an important environmental factor in pathogenesis of skin premature aging and skin cancer.[1] Excessive UVB radiation (with a wavelength of 290-340 nm) on skin can be dangerous and cause DNA mutation. Direct exposure of UVB can penetrate the basal cells of the epidermis, forming ROS (Reactive Oxygen Species) and triggering the formation of 8-hydroxy-deoxyguanosine (8-OHdG). 8-OHdG is the most potentially DNA damage and very mutagenic.[2] ROS cause oxidative damage to DNA and produce photoproduct 2-dipyrimidin. DNA photoproduct resulting from UVB is often called cyclobutane pyrimidine dimers (CPD) and (6-4) photoproduct.

In normal conditions, photoproduct will be repaired by the DNA nucleotid exicision repair (NER) but NER have limited capacity.[3] In addition to causing DNA photoproduct, UVB radiation can also induce cyclooxygenase-2 (COX-2) that play a role in chronic inflammatory processes and carcinogenesis.[4] UV radiation interfere with the immune system by

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destroying Langerhans cells and induce cytokines from keratinocytes or via macrophages.[5]

Bixin isolated from *Bixa orellana* L. seeds are some of pigments with the major components called Bixin ($C_{25}H_{30}O_4$) of approximately 80%.[6] Bixin has a double bond conjugation which has the ability to capture and absorb ultraviolet radiation. Bixin is a natural antioxidant that can prevent some types of cancer and other degenerative diseases.[6] Bixin from *B. orellana* L. has characteristic of cyclo-oxygenase (Cox)-1 inhibitors and Cox-2.[7] Cox-2 inhibition decreasing the prostaglandin biosynthesis and reducing the inflammatory response. Bixin is also effective as a Radical Oxygen Species (ROS) scavenger, which acts as a protective agent against free radicals.

In general, this present study is aimed at evaluating the effect of MJ protein as photoprotector against UVB irradiation and the chemoprevention againts skin cancer in vivo, especially to determine whether MJ protein posses protection against inflammation (inflammation-associated oedema) induced by UVB radiation; determine the potential of MJ protein to protect against UVB- induced immunosuppression and to reveal its effect on photoreceptor.

## Materials and methods:

Fresh seeds of *Bixa orellana* L. were collected from Salatiga, Indonesia in 2015. A voucher specimen was deposited in the Biological Laboratory of Universitas Islam sultan Agung, Semarang, Indonesia.

**Mice.** Fifty male BALB/c mice, aged about 6 weeks, were obtained from breeding colony of the Integrated Research and Testing Laboratory at the Gadjah Mada University. The mice were distributed into 5 experimental groups of 5 mice. They were kept under conventional animal house condition, 10 per cage, at room temperature. The mice were fed with standard laboratory mouse pellet and tap water ad libitum.

## Ethical Approval:

Approval by the Ethical Committee of Health and Medical Research, Universitas Islam Sultan Agung was obtained before these studies commenced.

### Bixin lotion preparation

The bixin lotion were made by mixing the crude extract with base lotion which consists of stearyl alcohol, vaselinum album, propilene glicol. The bixin lotion were topically applied at the dorsum of mice with the dose of 125, 2.5 and 0.05 µg.

### Pretreatment and treatment with Bixin lotion

All mice were shaved on the dorsal site and distributed into 5 groups of 5. Three groups of 5 mice received pretreatment with topical application of Bixin lotion for 10 days (once a day) with the dose of 125, 2.5 and 0.05 µg, in 0.1 mL volume, respectively. One group was pretreated with base lotion for negative control. The rest was allowed without any pretreatment for normal control.

The treatment regime comprised application of lotion immediately after each daily UVB exposure (days 1-3). Topical application of the lotion was then continued on days 4 and 5, i.e. for 2 days after the last irradiation.

### UVB irradiation.

Narrow band UVB was provided by 3 tubes of Ultraviolet-B Philips® 40W/12RS. The irradiance at the mouse dorsum was measured using an UV-meter, and recorded as 1 mWatt/cm$^2$ UVB. Mice were exposed on the dorsum, to a dose providing 360 mJ/cm$^2$ of UVB, which is approximately one minimum erythemal dose each day for three and five consecutive days to study UVB-induced inflammation and UVB-induced immunosuppression, respectively.

### Inflammation-associated edema

Edema was measured as the middorsal skinfold thickness, using a micrometer (Prohex, Germany), before and at 24 h intervals following irradiation. The average skinfold thickness was obtained at the peak of the response (at 48 h after irradiation) for each group and statistical significance between the different treatments was assessed using Tukey's test (SPSS 13.0 for Windows). The measurements were repeated numerous times with the same results of which a typical example is presented here.

### Inductions of CHS

The CHS response was induced by sensitization on the abdomen with 0.1 mL of freshly prepared 2% (wt/vol) oxazolone (Sigma) in ethanol on days 8 and 9 following treatment with UVB as previously described (Widyarini, 2001). Mice were challenged on day 15 by painting both outer and inner surfaces of each pinna with 5 µL freshly prepared 2% oxazolone. The maximum ear thickness was determined by measuring with the micrometer 18-20 later, and the average ear swelling was calculated as the difference from the average prechallenge ear thickness. The CHS responses were compared with mice that had not received UVB radiation, and the percentage suppression by the UVB was calculated as :

**%suppression =**

**100 - [average ear swelling in UVB mice] : [average ear swelling in non-UVB mice] x 100%**

Statistical significance between the different treatments was assessed using Tukey's test. The experiments were repeated at least twice with the similar results. As the control CHS reaction varies slightly between groups.

**Ethical Clearance**

All procedures performed involving animal were approved by *Komisi Etik Penelitian Kedokteran dan Kesehatan* (Medical and Health Research Ethics Committee) Faculty Medicine of Universitas Islam Sultan Agung.

**Results:**

**The Protection Againts UVB-induced Inflammation**

The effect of Bixin lotion treatment at various doses after exposure UVB for 9 minutes, causes the middorsal skinfold thickness at 24 hours, 48 hours and 72 hours after the first UVB exposure is different from that in untreated mice. The control with base lotion group (untreated mice) had a highest average middorsal skinfold thickness compared with the group of treated mice with various doses of Bixin lotion (Table 1).

**Table 1. The Average Middorsal Skinfold Thickness in Different Group**

| Group | The mean difference skinfold thickness (mm) after UVB exposure | | |
|---|---|---|---|
| | 24 hours | 48 hours | 72 hours |
| Base lotion | 0.87 | 1.63 | 1.84 |
| Bixin lotion 0.05 µg | 0.79 | 1.19 | 1.21 |
| Bixin lotion 2.5 µg | 0.41 | 0.97 | 1.05 |
| Bixin lotion 125 µg | 0.60 | 0.91 | 0.97 |

The trend of increase in middorsal skinfold thickness shows that the pattern is in the same direction with the exposure UVB but there is a qualitative difference among groups. The middorsal skinfold thickness was started at 24 hours following UVB irradiation, but the peak is occure at 72 hours. The mean difference of the middorsal skinfold thickness on 72 hours after UVB irradiation is highest for the base lotion group (control), then it is followed by the group treated with Bixin lotion 0.05 µg; 2.5 µg and125 µg respectively (Figure1).

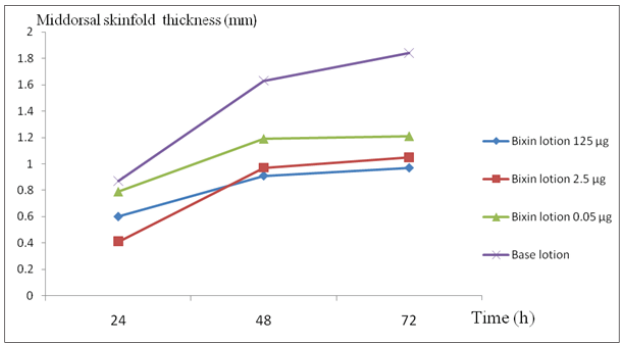


Figure 1. The trend of skinfold progress in different group

Fig 1. Profile of protective effect of Bixin lotion (125; 2.5 and 0.05 µg) against UVB-induced inflammation (UVB 360 mJ/cm$^2$, 1 x per day for 3 days), expressed as the average middorsal skinfold thickness in groups of five mice.

The oneway Anova analysis of the middorsal skinfold thickness of mice at 72 hours after UVB irradiation shows the significant differences in it among the groups ($p$=0,05), and the LSD Test toward all groups shows the significant differences between the base lotion (control) group and the all doses of Bixin lotion groups, nevertheless there is no significant differences among the various doses of Bixin lotion groups.

**The Protection Againts UVB-induced Immunoupression**

In this research, the protection to CHS supression was measured at 16 hours after UVB irradiation, when immunosupression at base lotion group (control) is maximum, meanwhile the Bixin lotion groups, have the maximal protection agains UVB-induced immunosupression. Imune respon was measure as contact hypersensitivity (CHS) respon described as the differences ratio of ear thickness after and before the challenge with oxasolon toward UVB irradiated and non irradiated mice. UVB radiation will reduce the immune respon, causing the decrease in the oxasolon challenge-ear edema. The decrease in edema as a evidence that bixin lotion offer a protection to UVB-induced immune supression.

This research finds that topical application of bixin lotion reducing the CHS respon. CHS respon at control group is 34,22% reduced to 11.4; 0.5 and -0% by Bixin lotion at the dose of 0.5 µg; 2.5 µg and 125 µg respectively (Table 2, figure 2). The result of dependent T test between mice with exposure UVB and no exposure UVB show the significant difference in control group and 125 doses group ($p<0.05$)

**Table 2. The protective effect of topical bixin lotion to CHS suppression induced by UVB**

| Doses | UVB | non-UVB | Immune Supression (%) |
|---|---|---|---|
| Bixin lotion 125 µg | 45.2 | 27 | -0 |
| Bixin lotion 2.5 µg | 20 | 20.1 | 0,5 |
| Bixin lotion 0.05 µg | 22.7 | 26 | 11,54 |
| Base lotion | 22.4 | 34 | 34,22 |

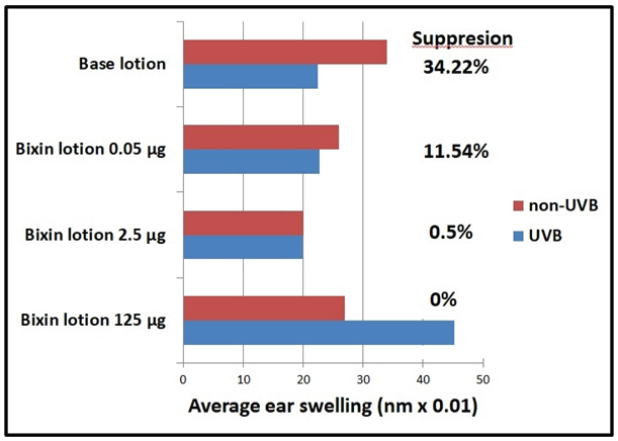


Figure 2. The protective effect of topical Bixin lotion (0.05-125 µg) to CHS supression induced by UVB, measured at at 16 hours after UVB irradiation. The data expressed as average ear swelling as a contact hypersensitivity (CHS) respon in a group containing 5 mice compared with the control (unexposured by UVB) mice. The result of dependent T-Test indicating the significant difference between mice with UVB exposure and no UVB exposure ($p<0,05$)

## Discussion

This study proves that bixin lotion can reduce the UVB-induced inflammation at the dose dependent manner. Even though, the mechanism of the reduction of inflammation was not elaborated in this research. According to the previous study, the possible mechanism of bixin lotion is pass through the cyclooxygenase (Cox) inhibitory. The bixin may inhibit the Cox enzyme resulting in the reduction of prostaglandin (PG)-$E_2$ synthesis. UVB radiation can exaggerate the Cox enzyme to increase $PGE_2$ synthesis that leading the process of inflammation along with the existence of erythema and sunburn.[8] Besides that, UVB radiation also causing DNA damage directly and indirectly. The damage on DNA results the skin cancer .[4]

The factor of immune also triggers the process of inflammation because of the exposure of UVB. UVB can activate the production of TNFα and *proinflammatory* sitokin agent such as IL-1, IL-6 which can increase the process of melanogenesis.[8] The further effect of the oxidative damage can generate DNA mutation and carcinogenesis.[9]

The result of this research is similar to previous study that shows that bixin isolated from *B. orellana L.* seeds has the effectiveness of antioxidant in quencher ROS.[10] The using of Bixin lotion in all dose range capable to minimize the skin inflammation on UVB irradiation mice. This antiinflammation effect also consistent with the previous in vitro study reporting that bixin isolated from *B. orellana* L seeds capable to inhibit Cox-1 and Cox-2 followed by reduction on PG synthesis and inflammation response.

The mechanism of Bixin lotion to reduce the inflammation and immune suppression induced by UVB has not confirmed from this research. But it is assumed that there are some mechanisms are involved, including the reduction of DNA damage, antioxidant pathway, SOD activity. In addition, it is possible that the bixin lotion also has an apoptotic induction mechanism which is related to the photoprotection effect as proved in the *Mirabilis jalapa* L leaf.[11] This needs to be clarified in further study.

## Conclusions :

It can be concluded that bixin lotion had potential to reduce the inflammatory edema reaction and the suppression of CHS of mice induced by UVB radiation. Because of the correlation between the inflammation and immonosuppression caused by UV and photocarcinogenesis, this study indicating that Bixin lotion may have a prospect as photo-protective agent in cosmetic and as chemopreventive agent in skin carcinogenesis.

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**Conflict of interest:** The investigators *declare that they have* no conflict of interests.

## Authors' Contributions:

Data gathering and idea owner of this study: Atina Hussaana

Study design: Atina Hussaana, Suparmi

Data gathering: Suparmi, Hani Afnita Murti

Writing and submitting manuscript: Atina Hussaana, Suparmi.

Editing and approval of final draft: Atina Hussaana, Hani Afnita Murti

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