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EFFECTS OF TOPICAL ADMINISTRATION OF *NYMPHAEAE LOTUS* FLOWER EXTRACT ON UVA-INDUCED PHOTOINFLAMMATION IN MICE

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KEYWORDS: Photoinflammation, UVA, *Nymphaeae lotus* Linn, Prostaglandin

INTRODUCTION

Nymphaeae lotus Linn. (water lily, NL) is an annual plant in the Nymphaceae family that grows in freshwater. Its trunk and bulb are in the soil under water. The flowers display in a wild variety of colors from pink, red, purple, yellow, and white. NL has a various pharmacological activities, such as antioxidation, anti-tyrosinase, hepatoprotection, and anti-inflammation (Debnath *et al.*, 2013). Skin exposure to ultraviolet (UV) light produces various types of biological effects, such as immunosuppression skin inflammation, skin cancer, and so on (Hruza and Pentland, 1993). Particularly, skin inflammation results in the mediation of prostaglandin E_2 (PGE_2). An increase in PGE_2 is the sign of UV-induced inflammation in the skin (Miller *et al.*, 1994). Therefore, the purpose of this study was to elevate whether NL could protect UV-induced skin inflammation in mice by determining both skin edema and PGE_2 levels. It might be beneficial for developing as a cosmeceutical product from plants.

MATERIALS AND METHODS

Materials Thirty male ICR mice were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Nakhon Pathom. Age of the mice at the beginning of study was 2 months old. They were acclimatized under standard conditions for 1 week with free access of food and water. All procedures and animal care were approved by Institutional Animal Care and Use Committee of Thailand Institute of Scientific and Technological Research.

Flower of NL was extracted with 95% w/v ethanol. It was prepared in cream base at 3% and 6% w/w. Cream base was also used as a vehicle. PGE_2, a primary mediator of inflammation, kit (Cayman Chemical, USA) was used to determine PGE_2 levels in the skin.

UV box was developed in our laboratory. It was made from wood in the size of 25x30x45 cm. The cover of the box had a small compartment (20x10x6 cm) on the top for carrying 3 UVA lamps. On/off switch was installed outside the box. UVA lamp (8 watts; Panasonics, Switzerland) was used to employ UVA approximately 200 mJ/cm^2.

Methods

UVA-induced photoinflammation Mice were shaved into 2x2 cm on the bottom back for one day before the experiment. On the day of experiment, mice were randomly divided into 5 groups (6 mice of each). The shaved skin of all mice, except one group was reserved to be a sham group, was exposed to the UVA light approximately 200 mJ/cm^2 for 30 min from directly above after 1 hr of topical administration of NL (3% and 6%, respectively) or cream base. The control group was exposed to the UVA light only, whereas the sham group was not treatmented with either cream or UVA. 48 hr after UV exposure, all animals were sacrificed. Exposed skin was removed. The thickness of the skin was measured at three midline sites using a pocket thickness gage (Mitutoyo, Japan).

PGE_2 assay The method of PGE_2 assay was modified from Yoshida *et al.* (2006). All skin removals were frozen in liquid nitrogen, after which were broken by crushing in a mortar on dry ice. The skins were then homogenized in buffer containing 50 mM Tris-HCl and 0.25% sucrose (pH 8.3), and were centrifuged at 8,000 g (4°C) for 20 minutes. The supernatants were then collected and again centrifuged at 100,000 g for 20 min (4°C). The pellets were purified with SPE (C-18) according to the manufacturer's instructions of a PGE_2 ELISA kit.

Statistical analysis All data were expressed as mean ± standard error. Statistical analysis for comparing treatment effects of each group of mice was done by one-way ANOVA. Comparisons among groups were conducted using the LSD post-hoc analysis. Student's t-test techniques were also used. Statistical significance was defined as $p<0.05$.

RESULTS

UVA-induced photoinflammation The skins that were only exposed to UVA light (the control group) significantly increased 25% edema compared to non-exposed skins (the sham group) as shown in Figure 1 and Table 1. Pretreatment with either cream or NL was beneficial for skin photoinflammation. Cream base and both concentrations of NL cream significantly depleted skin edema induction by UVA irradiation (Figure 1).

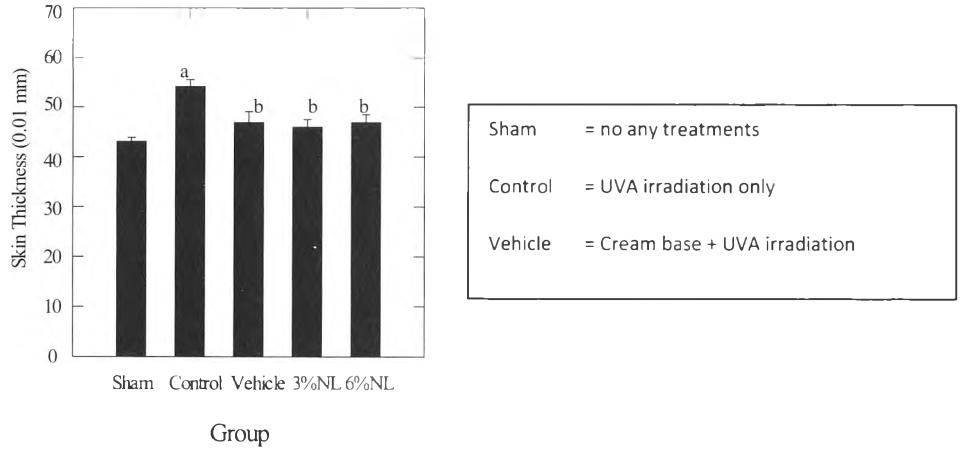

Figure 1 Effects of topical pretreatment with NL (3% and 5% w/w in vehicle) on UVA-induced photoinflammation. Skin thickness was determined and expressed as mean ± standard error. [a] $p<0.05$ relative to the sham group; [b] $p< 0.05$ relative to the control group.

Table 1 Effects of topical administration of NL (3% and 5% w/w in vehicle) on UVA-induced photoinflammation. Skin edema was shown in percents, whereas Sham = no any treatments, Control = UVA irradiation only, Vehicle = Cream base + UVA irradiation, 3%NL = 3%w/w NL + UVA irradiation, and 6%NL = 6%w/w NL + UVA irradiation

Group	Skin edema (%)
Sham	-
Control	25.5
Vehicle	8.8
3% w/w NL	6.7
6% w/w NL	8.8

PGE_2 assay The levels of PGE_2 in the skin samples were shown in Table 2. The skins which were only exposed to UV irradiation (the control group) exhibited the highest amounts of PGE_2. They were approximately 2-folds higher (98.3 ± 33.6 pg/ml) than those seen in non UV-exposed mice (45.0 ± 9.2 pg/ml; the sham group). As displayed in Table 2, the increase in UVA-induced PGE_2 levels was significantly diminished by pretreatment with both 3% and 5% w/w of NL. Particularly, 3% w/w NL exhibited a significant reduction of PGE_2 levels ($p<0.028$).

Table 2 Effects of topical administration of NL (3% and 5% w/w in vehicle) on UVA-induced PGE_2 levels. Sham = no any treatments, Control = UVA irradiation only, Vehicle = Cream base + UVA irradiation, 3%NL = 3%w/w NL + UVA irradiation, and 6%NL = 6%w/w NL + UVA irradiation.

Group	PGE_2 levels (pg/ml)	p-value	p-value
Sham	45.0 ± 9.2	1.000	
Control	98.3 ± 33.6	0.067	1.000
vehicle	83.3 ± 23.7	0.177	0.591
3% w/w NL	33.2 ± 8.2	0.670	*0.028*
6% w/w NL	49.4 ± 11.8	0.873	0.090

DISCUSSION

Skin inflammation could be produced by UVA irradiation since the skin thickness was found approximately twice increase compared to non-irradiated skin. Pretreatment with either cream base or NL remarkably shielded the skin from photoinflammation. However, the amounts of PGE_2 were found a significant decrease in only 3%w/w NL mice. Therefore, the data demonstrated that 3% w/w NL reduced UVA-induced skin inflammation probably involved in the decrease of this primary mediator of inflammation.

CONCLUSION

As a remarkable evidence to diminish the effect of UVA-induced photoinflammation found in 3% w/w NL mice on both investigations of skin edema and PGE_2 level, it suggested that pretreatment with 3% w/w NL possibly beneficiated for UV-exposed skin.

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