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Original Article

Protective Effects of Rapeseed Flower on Ultraviolet B-induced Skin Photoaging in Rats

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ABSTRACT

Background and Aim: Photoaging is a chronic ultraviolet (UV)-induced damage, attracting in skin appearance, which is so important in the field of cosmetic dermatology. Recently, antioxidants involved in Rapeseed flower and its products have been considered one of the readily accessible sources and might have beneficial implications on the photoaging. This study was designed to investigate protective effects of Rapeseed flower extract on UVB-induced skin photoaging *in vivo*. **Methods:** The plant flower was extracted with aqueous ethanol (90%). Male Wistar rats were divided into five groups of 10 animals each. Each group was administrated the extract in different doses and irradiated UVB radiation for 4 weeks. The degree of protection was quantified through the skin antioxidant enzyme activities including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), as well as the contents of glutathione (GSH), malondialdehyde (MDA) and hydroxyproline (HP). **Results:** Rapeseed flower extract inhibited the UVB-induced photoaging on rat skin by increasing SOD, GSH-Px, CAT, GSH, HP and decreasing MDA levels significantly compared to control group. **Conclusion:** Collectively, these data indicated that Rapeseed flower extract could be used as a safe and natural antioxidant for the protection of UVB-induced skin photoaging *in vivo*.

Key words: Antioxidant, Rapeseed flower extract, Skin photoaging, Ultraviolet radiation, Oxidative stress.

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INTRODUCTION

Ultraviolet radiation, which comprises wavelengths that are less than 400 nm, is involved in solar radiations. Among these radiations, the ultraviolet C (UVC, 100–290 nm) is absorbed and stopped by the ozone layer so we can consider that UVC is not implicated in skin photodamage. Otherwise, the Ultraviolet A (UVA, 320–400 nm) and the ultraviolet B (UVB, 290–320 nm) may induce epidermal and dermal damages by producing direct cellular DNA damage and also increasing oxidative damage of protein, lipid and DNA through the generation of reactive oxygen species (ROS). Particularly, UVB has strong cytotoxic and mutagenic effects and the immediate damaging of UVB on the skin could be 1000 times than that of UVA.[1,2] ROS can result in structural and functional alterations in the extracellular matrix (ECM) and also direct deleterious chemical modifications to cellular components, including DNA, proteins, lipids, which contributes to photoaging.[3] Rapeseed (*Brassica napus* L.), also known as rape, oilseed rape, rapa, rappi, rapaseed, is a bright yellow flowering member of the family Brassicaceae (mustard or cabbage family). The fatty acid composition of oil of the zero erucic acid commercial *Brassica napus* L. is typical for this species. It is rich in oleic acid and contains moderate levels of linoleic and linolenic acid. Rapeseed is grown for the production of vegetable oil for human nutrition in most countries worldwide. Rapeseed is also cultivated for the economic benefits such as oil-rich seed, biodiesel and animal feed in DPR Korea.

Few studies have reported biological components of Rapeseed pollen for the treatment of some diseases,[4,5] but there has been no research to use its flower as herbal medicine for the prevention and improvement of UVB-induced photoaging *in vivo*. Therefore, the present study prepared Rapeseed flower extract and evaluated their protective effects on rat skin photoaging induced by UVB irradiation, which could support utilization of this extract as a skin care agent.

MATERIALS AND METHODS

Preparation of the Ethanol Extract from Rapeseed Flowers

Rapeseed (*Brassica napus* L.) flowers samples collected from southern Huanghea province, DPR Korea in the spring season (April to May). The samples were authenticated and demonstrated that total flavonoid was several times greater than in the other area by National botanical institute of DPR Korea and extracted by Traditional Medicine Centre

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of Pyongyang University of Medical Sciences. The extract of *Brassica napus* L. flower was prepared using ethanolic method: 3 kg of dry flowers including pollen were immersed in 3L of 90% ethanol with intermittent shaking for 24hr, and then refluxed for 3h by heating. The filtrate was evaporated below 45°C under reduced pressure. The residue (yield: 9.1%) was designated as an alcoholic extract. The extract was quantified by a HPLC assay to contain main components: total flavonoid at 9.4%.

Animals

Male adult Wistar rats (11-12 week old, 250~300g) were provided by Laboratory Animal Centre of the Pyongyang University of Medical Sciences. All rats were housed in a stainless steel cage (50×40×60cm) at 24±1°C under a 12hr light-dark cycle with free access to food and water through the experiment, and were allowed to acclimate for two weeks before the start of experiment. Food intake and body weight were measured thrice and once a week, respectively. The Institutional Animal Care and Use Committee of the Pyongyang University of Medical Sciences approved the animal protocol used in this study.

Diet

After 2 weeks of acclimation, the rats were randomly divided into 4 groups (n=10): normal group (NG): rats fed the control diet only; model group (MG): rats fed the control diet only; RG-1: rats fed a diet containing 30mg/kg extract; RG-2: rats fed a diet containing 60mg/kg extract; RG-3: rats fed a diet containing 90mg/kg extract. The animals in each group were fed their experimental diets from the first day of UVB irradiation for 4 weeks and were irradiated with the same UVB source, except the normal group.

Ultraviolet-B Irradiation

After 1 week of acclimatization to the cage, the rat back was denuded using sulfureted sodium (8%, Jinshan Co. Ltd., Chengdu, China) over the depilation area of 16cm^2. All rats, except the normal group, were irradiated with the same UVB source.

FS40T12/UVB sunlamps (Philips, Amsterdam, The Netherlands) were used as a UVB source and the distance from lamp to dorsal skin was 20cm and intensity of irradiation was 80mW/cm^2. The procedure for UVB irradiation was done for 5 min twice a day during 4 weeks.

Skin Antioxidant Enzyme Activity Estimation

Rat back skin (about 1cm^2) was homogenized (10000rpm, 20s) with a Teflon glass tissue homogenizer (Remi, India) in cold normal saline. The homogenate (10%) was centrifuged at 3000rpm for 20min at 4°C, and total supernatant was used for an assays. Skin tissue superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, catalase (CAT) activity, glutathione (GSH) and malondialdehyde (MDA) contents were determined with the following methods. SOD was assayed according to the previous method.[6] To 0.5mL of skin homogenate supernatant, 0.5mL of 0.6mM EDTA solution and 1mL of 0.1M carbonate-bicarbonate (pH-10.2) buffer were added. The reaction was initiated by the addition of 0.5mL of 1.8mM epinephrine (freshly prepared) and the increase in absorbance at 480nm was measured. GSH-Px was assayed by the previous method with slight modification.[7] The reaction mixture consisted of 0.2mL of 0.8mM EDTA, 0.1mL of 10mM sodium azide, 0.1mL of 2.5mM H_2O_2, 0.2mL of GSH, 0.4mL of 0.4M phosphate buffer pH 7.0, and 0.2mL of skin homogenate supernatant and was incubated at 37.8°C for 10min. The reaction was arrested by the addition of 0.5mL of 10% Trichloroacetic acid (TCA) and the tubes were centrifuged at 2000rpm. To the supernatant, 3.0mL of 0.3mM disodium hydrogen phosphate and 1.0mL of 0.04% dithionitrobenzoic acid (DTNB) were added and the colour developed was read at 420nm immediately. The activity of GSH-Px was expressed as µmoles of glutathione oxidized/min per mg protein. CAT was assayed by the following method. To 1.2mL of 50mM phosphate buffer pH 7.0, 0.2mL of skin homogenate supernatant was added and reaction was started by the addition of 1.0mL of 30mM H_2O_2 solution. The decrease in absorbance was measured at 240nm at 30s intervals for 3min. The enzyme blank was run simultaneously with 1.0mL of distilled water instead of hydrogen peroxide.

GSH Estimation

GSH content in the supernatant was measured by reaction with DTNB. To 0.1mL of skin homogenate supernatant, 2.0mL of 0.6mM DTNB and 0.2M phosphate buffer (pH 8.0) were added to make up to a final volume of 4.0mL. The absorbance was read at 412nm against a blank containing TCA instead of sample. A series of standards treated in a similar was also were run to determine the glutathione content. 5-Sulphosalicylic acid was used to prevent oxidation of glutathione. The amount of glutathione was expressed as µmol/mg skin tissue.

MDA Estimation

MDA was determined by the thiobarbituric acid (TBA) method, based on its reaction with TBA to form thiobarbituric acid-reactive substances (TBARS). Determination of TBARS in the rat skin was measured as described previously with slight modification.[8] To 0.2 ml of skin homogenate supernatant, 0.2 mL of 8.1% sodium lauryl sulphate and 1.5 ml of 20% acetic acid solution (pH adjusted to 3.5 with sodium hydroxide) were added. Then 1.5 ml of 0.8% aqueous solution of TBA was added. The mixture was made up to 40mL with distilled water and heated in a water bath at 95°C for 60 min. In cooling water 1.0 ml of distilled water and 5.0 ml mixture of n-butanol and pyridine (15:1 v/v) were added. After centrifugation at 4000 rpm for 10 min. Absorbance of the organic layer was read at 532 nm. The level of MDA was expressed as nmol/mg of tissue. Protein concentration of skin tissue was measured using the method of Lowry and others,[8] using bovine serum albumin as standard.

Hydroxyproline Estimation

Equal amount of protein (0.1mg) from each sample of skin homogenate was mixed with an equal volume of 4N NaOH and hydrolyzed by autoclave for 20 min. The autoclaved sample was neutralized by an equal volume of 2N HCl. Chloramine T (0.056 M in 10% n-propanol and acetate citrate buffer) was added to oxidize the hydrolyzate for 25 min at room temperature. Ehrlich's aldehyde reagent (1 M p-dimethylaminobenzaldehyde in n-propanol/perchloric acid 2:1 vol/vol) was freshly prepared and added to develop the chromophore by incubating the samples at 65°C for 20 min. HP content was read at 550 nm. The assay was carried out in duplicate for each sample. The amount of HP was determined by comparison with a standard curve prepared from known concentrations of HP.[9]

Statistical Analysis of Data

Results were expressed as the mean and SEM. Data was analyzed by one-way analysis of variance (ANOVA) using SPSS 16.0 and the differences between the mean assessed using Duncan's multiple range test. P value of < 0.05 was taken as the level of statistical significance.

RESULTS

As shown in Table 1, skin antioxidant enzymes were destroyed by UVB irradiation and the activities of SOD, GSH-Px and CAT in MG rats significantly decreased compared to NG rats ($P<0.05$). Rapeseed flower extract could protect the SOD, GSH-Px and CAT against the damages

Table 1: Effect of Rapeseed flower extract on SOD, GSH-Px, CAT activity on photoaging rat skin.

Groups	SOD (U^A/mg Protein)	GSH-Px (U^B/mg Protein)	CAT (U^C/mg Protein)
NG	356.4 ± 10.3[a]	39.5 ± 2.0[a]	6.2 ± 0.9[a]
MG	168.5 ± 7.7[b]	20.4 ± 1.5[b]	2.5 ± 0.2[b]
RG-1	201.0 ± 9.40[c]	24.7 ± 0.7[c]	3.9 ± 0.4[c]
RG-2	234.1 ± 5.9[d]	25.8 ± 1.1[c]	4.2 ± 0.4[c]
RG-3	276.3 ± 9.8[e]	30.2 ± 1.9[d]	4.9 ± 0.7[d]

Each value represents the mean ± SEM of 10 rats per group. Means in the same column not sharing the same letters are significantly different (P<0.05).

[A]One unit of SOD was defined as the amount of the enzyme inhibiting the epinephrine autoxidation by 50%.

[B]One unit of glutathione peroxidase was defined as the amount of enzyme leading 1μmol of GSH oxidized per min.

[C]One unit of catalase activity was defined as the amount of enzyme that reduces 1μmol of H_2O_2 per second.

Table 2: Effect of Rapeseed flower extract on GSH, MDA concentration on photoaging rat skin.

Groups	GSH (μmol/mg Protein)	MDA (nmol/mg Protein)
NG	92.8 ± 2.6[a]	4.5 ± 0.4[a]
MG	42.8 ± 0.8[b]	9.20 ± 0.9[b]
RG-1	68.6 ± 1.7[c]	6.9 ± 0.5 [c]
RG-2	71.9 ± 1.2[c]	6.4 ± 0.6[c]
RG-3	77.2 ± 2.6[d]	5.4 ± 0.4[d]

Each value represents the mean ± SEM of 10 rats per group. Means in the same column not sharing the same letters are significantly different (P<0.05).

in dose-dependent manners, as depicted by the significantly increase of enzyme activities in all RG groups.

Table 2 showed that UVB irradiation caused a significant decrease in GSH contents in MG rats (P<0.05). Meanwhile, MDA contents in MG rats significantly increased compared to NG rats. Giving the extract, the decrease of GSH and increase of MDA was significantly inhibited compared to MG group (P<0.05) and the inhibitory effects were in dose-dependent manners.

As shown in Figure 1, UVB-irradiation to rats significantly decreased HP content in skin (MG). However, RE could protect the skin HP from the UVB-induced damage (P<0.05).

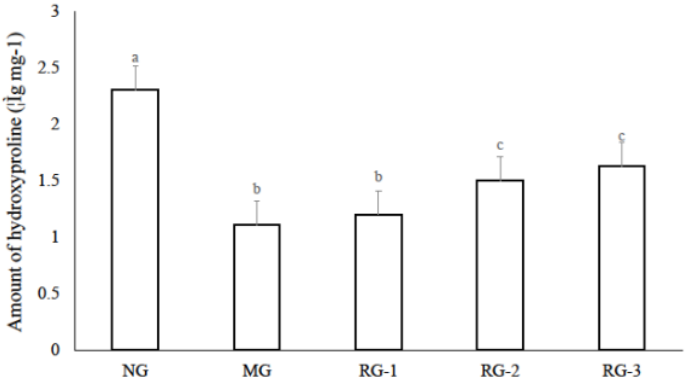

Figure 1: Changes in amounts of hydroxyproline in photoaging rat skin.

DISCUSSION

Oxidative stress including UVB-induced skin damage is attributable to the formation of reactive oxygen species (ROS) and although cytoplasmic and mitochondrial defence mechanisms including the antioxidant enzymes like GSH-Px, SOD, and CAT have evolved to quench ROS, these antioxidant defence enzymes of cell may become overwhelmed.[10] Medicinal plants contain many types of effective antioxidants, mostly phenolic acids, flavonoids and carotenoids.[11] Most of studies focus on external free radical scavengers and antioxidants of botanical origin such as tea polyphenols, β–carotene, etc. at cellular oxidative stress and previous studies reported the efficacy of these agents for many kinds of oxidative stress *in vitro* and *in vivo*. Also, we believed that botanical extracts including radical scavengers or antioxidants could alleviate oxidative stress and improve the function of endogenous antioxidant enzymes due to scavenging ROS, especially could protect skin injury and aging acceleration by UV-induced oxidative stress. In DPR of Korea, some people use Rapeseed flowers and their leaves as the recipe for treatment of diseases from half a century ago and exports demonstrated that Rapeseed flowers and their extract involve several antioxidants: flavonoid and flavonol aglycone including quercetin, kaempferol and isorhamnetin. This shows that Rapeseed flowers could be a potentially important source of dietary flavonols, in particular, quercetin and kaempferol known as strong antioxidants. Therefore, at present study we used Rapeseed flowers as antioxidant for the improvement of skin damage by UV-induced oxidative stress. Total flavonoid content of prepared flowers extract was at 9.4% and animals in RG group were fed diet containing extract from the first day of UVB irradiation for 4 weeks. Our investigation showed that severe oxidative stress induced by UV irradiation in rat skin was accompanied by the notable decrease in activities of SOD, CAT, GPH-Px, as well as GSH content and increase in MDA level. However, treated with Rapeseed flower extract, the decreases in the enzyme activities were significantly prevented, showing that the extract could exert favourable effects against pathologic alterations induced by UV irradiation. The protective effects on the enzyme activities might be contributed to the antioxidative properties *in vivo*, as the extract with flavonoid exhibited the strong antioxidant activity. Endogenous reducing power plays a crucial role to improve the UV-induced damages. GSH is an intracellular low-molecular-weight antioxidant, acts as a protective agent scavenging ROS in physiologic processes through the redox mechanism. The depletion of GSH in skin makes it sensitive to UV-induced mutations and cell death.[12] MDA is the product of lipid peroxidation and usually quantified to estimate the lipid peroxidation. UV exposure of rat skin caused the obvious decrease in GSH contents and increase in MDA levels, indicating that UV irradiation exhausted the endogenous reducing power and catalysed the lipid peroxidation, likely via pathways involving ROS. However, the GSH contents in the RE-administrated groups (RG-1, 2, 3) increased significantly compared to non-administrated group (MG). The actions might be explained by the antioxidant role of Rapeseed extract to suppress ROS formation, inhibit propagation of lipid-peroxidative chain reactions, and thus result in the decrease of MDA levels. The results were similar to the previous research. Lipid is particularly important in maintaining the permeability barrier function. An altered lipid organization may weaken the skin barrier function.[3] Chronic and high dose of UV exposure markedly reduced the crude lipid content per skin weight in our study. The reason might be that ROS UV-induced aggravated lipid peroxidation of rat skin. Collagen, a fibrous protein of the ECM, is a major constituent of connective tissues such as skin, tendon, ligament, cartilage, and bone. The functional properties of skin depend on the integrity of collagen in the dermis. Repeated exposure to UV radiation leads to alterations in skin composition and its mechanism has been reviewed.[13] ROS activate

cell surface receptors including epidermal growth factor receptor, leading to intracellular signalling. Expression of nuclear factor AP-1 is induced by activated kinases or ROS themselves. Increased AP-1 transcription and its activity interfere with the effect of transforming growth factor-β, suppressing collagen gene expression and activating keratinocyte proliferation. Such damage to the skin is believed to lead to visible changes. UV-induced ROS could induce lipid peroxidate in membranes of primary fibroblast cells, degenerate fibroblasts in dermis, and consequently decrease the content of skin collagen. According to our research, Rapeseed flower extract could increase the HP content in rat skin in dose-dependent manners but higher dose of 60mg/kg showed the significant effects. There have been some reports that involves much increase of HP content in photoaged skin *in vivo* with applying collagen substances.[14,15] This suggested that co-use of this extract and collagen can exert much higher effect on HP increase and further studies are needed to clarify the mechanisms responsible for the inhibitory effect of Rapeseed flower extract and collagen on photoaging.

Limitations of the Study

There are several members of family Brassicaceae, but we have tested the antioxidant effects of only one member, *Brassica napus* L., so further studies are required for other members in the future.

CONCLUSION

Rapeseed flower extract as external antioxidant significantly increased the activity of the antioxidative enzymes, efficiently suppressed the lipid peroxidation and protected the collagen from the UV irradiation in the rat skin. Our study demonstrated that Rapeseed flower extract could be used one of the efficient agents for prevention and improvement of skin aging induced by UV irradiation.

ABBREVIATIONS

UV: Ultraviolet; **ROS:** Reactive Oxygen Species; **ECM:** Extracellular Matrix; **SOD:** Superoxide Dismutase; **GSH-Px:** Glutathione Peroxidase; **CAT:** Catalase; **MDA:** Malondialdehyde; **HP:** Hydroxyproline; **DTNB:** Dithionitrobenzoic Acid; **TBA:** Thiobarbituric Acid; **TBARS:** Thiobarbituric Acid-Reactive Substances.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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