Original Article

Efficacy of Sweet Pumpkin in Relieving Contact Dermatitis in Chronically Stressed Rats

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Abstract

Background: Contact dermatitis (CD) is considered among the common inflammatory skin diseases worldwide. *Cucurbita moschata* Duchesne has antioxidant, anti-inflammatory, and antidepressant activity beside many other beneficial effects. **Objectives:** This study aimed to assess the effect of pumpkin fruit extract in treating CD in mice exposed to chronic stress and to explore the mechanism through which pumpkin can relief these changes. **Materials and Methods:** Thirty male albino rats were divided into three groups ($n = 10$); the control and two experimental groups that were exposure to chronic unpredictable mild stress for 4 weeks then painting with 1-fluoro-2,4-dinitrofluorobenzene (DNFB) for 3 consecutive days/week for 2 weeks to induce CD. Biochemical assessment of corticosterone level and antioxidants activity was performed. Skin of affected areas was excised, processed for histopathological examination. **Results:** DNFB-induced CD presented with dryness, hardness, and scaling. There was a significant reduction ($P < 0.001$) in the levels of superoxide dismutase, glutathione peroxidase and catalase activity in the skin of rats had CD. Histopathologically, the shin showed hyperplastic-thickened epidermis, focal elongation of the rete ridges, inflammatory cells infiltration in the superficial dermis, and increased collagen fibers. Local administration of pumpkin extract significantly increased the antioxidants activity in the skin and alleviated the CD-associated changes. **Conclusions:** This study showed that the pumpkin fruit extract could have a potential in treating CD in stressed conditions mainly via its enhancement of skin antioxidant activity.

Keywords: Antioxidants, chronic stress, contact dermatitis, corticosterone, pumpkin, superoxide dismutase

Introduction

Cucurbita moschata Duchesne (sweet pumpkin), the commonly cultivated vegetable worldwide, is rich in flavonoids, phenolics, vitamins, carbohydrates, and amino acids.[1] Sweet pumpkin has a wide range of biological activities included anti-inflammatory, antifatigue, antioxidant, anticarcinogenic, antimicrobial, and anti-obesity activity.[2] Skin, the largest organ in the body, is the first line of defense and acts as a barrier against pathogens via its immunologic elements. It also has a role in protection from chemical injury and ultraviolet (UV) radiation, temperature regulation and sensory perception, excretion, and synthesis of Vitamin D.[3]

Psychological stress adversely affects the immune system and aggravates various skin diseases, such as psoriasis, alopecia areata, and atopic dermatitis.[4] Contact dermatitis (CD) occurs when skin is exposed or come into contact with a particular irritant substance that directly damages the outer layer of skin.[5] These substances include volatile organic compounds such as benzene, xylene, and formaldehyde, which are considered the main cause of skin irritation.[6]

Dermatitis is considered worldwide common inflammatory skin disease that affects millions. It includes CD and psoriasis.[7] CD is commonly presented with erythema, swelling, papules, and bullae. Microscopically, it is characterized by increased proliferation of keratinocytes, increased dermal vascularity, and dermal and epidermal inflammatory infiltrate.[8] Experimental work carried out by Lyu *et al*. on mice model of CD proved that painting the skin with gasoline

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derivatives (1-fluoro-2,4-dinitrofluorobenzene (DNFB) resulted in inflammatory skin reaction.[9]

Medical therapy of skin diseases such as eczema (nonspecific dermatitis) includes topical treatment with anti-inflammatory such as topical corticosteroids in the form of creams or lotions.[10] Some cases need systemic treatment such as antibiotic and antihistamines or oral corticosteroids.[11] The aim of all these medical therapies are minimizing pruritus, improving skin barrier integrity, increase the skin hydration, and decrease inflammation and dryness.[12] Unfortunately, the use of such medical therapies is usually associated with many side effects. Therefore, the demand for natural remedies is rising in developing countries, as natural substances may be effective, safe, and cheap.[13]

C. moschata Duchesne (synonym *Pepo moschata*), also known as pumpkin, belongs to the family *Cucurbitaceae*. It is a widely distributed plant mainly used for its fruit and seeds.[14] In a recent study, Sweetme Sweet Pumpkin™, a baked form of *C. moschata* Duchesne with several health benefits, showed an efficacy in treating patients with depression in Korea.[15] Although the pumpkin oil was proved to have a promising effect on healing wounds in animal assays, its role in relieving the CD in term of pathological changes, specifically in stressful conditions have not been previously studied.[16] Therefore, this study aimed to assess the effect of pumpkin fruit extract in treating CD in mice exposed to chronic stress and to explore the mechanism through which pumpkin can relief these changes.

Materials and Methods

Extraction of pumpkin *Cucurbita moschata* Duchesne

Fresh pumpkin (*C. moschata* Duchesne) fruits were obtained from the local market at Jeddah, Saudi Arabia. Extraction of (*L. Cucurbita pepo*) was prepared as was previously described by Wang *et al*.[2] The raw fruits with skin were cut with a slicer dried by lyophilize machine freeze-drier and then crushed by electrical machine. The obtained dried powder (50 g) was mixed with 450 mL of 80% ethanol for 1 day at 37°C temperature, left it in shaker machine for 1 day, and filtered with cotton and filter paper at 2nd day. This extraction process was repeated twice, and the excess solvent was evaporated under reduced pressure using a rotary vacuum evaporator to give an ethanol extract. It was left at fume hood to extra evaporation for ethanol then the extract was dried in freeze-drier machine.

Preparation of pumpkin cream for topical use

Simple ointments of ethanolic extract of liquid chromatography were formulated in Vaseline at a proportion of 2% (w/w) using a ceramic mortar and pestle and kept in sterile container.

Experimental groups and dosage

Thirty male albino rats weighing from 150 to 200 g obtained from the animal house of the King Fahd Medical Research Center were utilized in this study. The rats were acclimatized in the laboratory conditions for 1 week, before starting the experiment. They were housed in plastic cages in an air-conditioned room at 22°C ± 1°C, offered the standard animal chow and water *ad libitum*. Ten rats were not exposed to any procedure and left as control group (GI). The other twenty rats were subjected to the procedure of chronic unpredictable mild stress (CUMS) for 4 weeks as was previously described.[17] After completing the 4 weeks, blood samples were taken and serum corticosterone level was assessed to confirm induction of stress.

At the start of the 5th week, a rectangular area of 3 cm × 2 cm on the dorsal surface of the rats was marked and shaved with an electrical shaving machine. This area was exposed to DNFB (0.1%, v/v) in acetone: Olive oil 4:1 (AOO) for 3 consecutive days in order to induce CD, according to Kim *et al*.[15] Four days later, each mouse was painted onto the dorsum with 30 µL of DNFB (0.2%, v/v) in AOO every 2 days for 15 days. The painted areas were observed for 2 weeks. The rats were then divide into two groups (n = 10 each); GII group treated with the vehicle (AOO) used in sensitization while GIII group treated with pumpkin extract (PE). Rats of the latter group were locally treated with PE at a dose of (0.52 µl/mm^2) using special paintbrush for 2 weeks according to Bardaa *et al*.[16]

Biochemical techniques

Blood samples were obtained for biochemical assessment from the intra-orbital sinus during the experiment and from the heart at the end of the experiment. Centrifugation was performed at 3000 rpm for 15 min at 4°C to obtain the serum from the blood samples and was kept at −18°C. Samples of the affected skin were obtained and kept at −80°C for assessment of protein expression.

Corticosterone level (ALPCO Diagnostics, Orangeburg, NY, USA) was assessed in the serum during and at the end of the experiment using ELISA kits according to the manufacturers' instructions.

Superoxide dismutase (SOD) catalyzes the dismutation of the superoxide radical anion into hydrogen peroxide and molecular oxygen. To determine the SOD activity, nitroblue tetrazolium (NBT) was used. SOD Assay Kit (Biodiagnostic; Egypt) allowed very convenient SOD assaying through reduction of NBT to insoluble blue formazan. The method described by Pou *et al*.[18] was used.

To quantify the catalase (CAT) activity, a calibration curve was generated for the assay and all samples, using Biodiagnostic; Egypt assay kits. The method described by Gamal *et al*.[19] Glutathione peroxidase (GPx) kit (Randox Labs, Crumlin, UK) was used where GPx catalyzes the oxidation of glutathione with cumene hydroperoxide. The method described by Gamal *et al*. was used.[19]

Histological techniques

After 2 weeks of treatment, rats were anesthetized with ether then decapitated. The blood was obtained from the heart after opening the chest wall. Skin samples of 5 mm × 5 mm from

the affected area were dissected out and fixed in 10% neutral buffered formalin to be further processed for obtaining of paraffin blocks. Paraffin sections at 4-µm thickness were prepared and stained with hematoxylin and eosin (H and E) and Masson trichrome for collagen fibers. In addition, another set of paraffin sections at same thickness was immunohistochemically stained using the streptavidin–biotin–peroxidase technique.

Olympus microscope BX-51 (Olympus) connected to a digital camera and a computer was used for photographing. Pro Plus image analysis software was used for morphometric assessment of the skin section. Measurement of the epidermal thickness was carried out through a perpendicular line drawn from the basal layer to the end of the granular layer. At least five fields from each slide were examined and the mean was calculated.

Quantitative data were statistically analyzed using the Statistical Package for the Social Science Program (SPSS, SPSS Inc., Chicago, Illinois, USA) Version 20 and presented in the form of mean ± standard deviation. The *F* test (one-way analysis of variance) was used to compare the studied groups. *Post hoc* test with the least significant difference test was used to compare between two groups. $P < 0.05$ was considered as significant.

Results

Biochemical results

Corticosterone level was assessed at the end of the first 4 weeks of the experiment after completion of the exposure to the CUMS procedure in order to confirm the occurrence of stress. It was found that exposure to CUMS significant increased ($P < 0.001$) basal serum corticosterone levels in both experimental groups (II and III) compared to the control group [Table 1].

Antioxidants profile was assessed at the end of the experiment in the skin areas painted with the DNFB alone (GII) or treated with PE (GIII). It was noticed that there was a significant reduction ($P < 0.001$) in the levels of SOD, GPX, and CAT in GII compared to the control. On the other hand, following local administration of PE to the affected skin area the levels of SOD ($P = 0.04$), GPX ($P = 0.01$), and CAT ($P = 0.07$) were elevated in GIII compared to the untreated group (GII) [Table 1].

Gross changes in the skin

When the gross morphology of the dorsal skin of the rats was examined through the experiment, it was noticed that the skin of the control rat had normal appearance. After 2 weeks of painting with DNFB, rats of GII showed dryness, hardness, and scaling of the painted area, indicating occurrence of CD [Figure 1a and b].

Application of PE on the affected area started from the 3rd week and continued during the 3rd and 4th weeks. It was noticed that PE progressively decreased dryness and scaling. By the end of the 4th week, dorsal skin of GIII showed marked improvement of the DNFB-induced skin changes [Figure 1c and d].

Histological results

H and E-stained sections of the dorsal skin of albino rats were prepared and examined to assess the histopathological changes induced by DNFB on the skin. It was observed that skin of control rats of gastrointestinal (GI) was intact thin epidermis, superficial dermis, and deep dermis [Figure 2a and b]. Skin of rats of GII that were painted with DNFB for 2 weeks showed hyperplastic thickened epidermis, focal elongation of the rete ridges was also observed together with marked increased thickness of the granular layer. The superficial dermis showed inflammatory cells infiltrating [Figure 2c and d]. Skin of some

Table 1: Levels of serum corticosterone and antioxidants in the skin of the studied groups

	Group I	Group II	Group III
Corticosterone	6.1±1.3	10.9±1.8	10.3±2.8
		P1<0.001	P1<0.001
SOD in skin	4.11±1	1.74±0.67	2.65±1.02
		P1<0.001	P2=0.04
GPX in skin	58.93±5.2	35.7±12.5	46.02±9.1
		P1<0.001	P2=0.01
CAT in skin	116.7±10.1	88.9±17.6	100.55±15.5
		P1<0.001	P2=0.07

P1: Significance versus the Group I, P2: Significance versus Group II. SOD: Superoxide dismutase, CAT: Catalase, GPX: Glutathione peroxidase-1

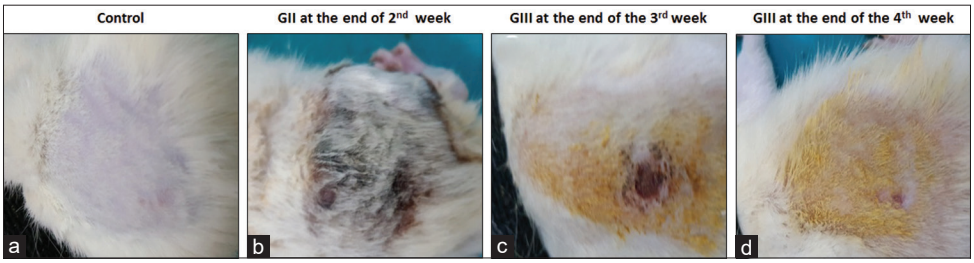

Figure 1: Gross appearance of rat dorsal skin during the experiment. (a) G1: Control with normal skin appearance. (b) GII: (1-fluoro-2,4-dinitrofluorobenzene-painted) after 2 weeks showing marked affection in the form of dryness and scaling. (c) GIII: (pumpkin extract-treated) rat after 1 week of treatment (end of the 3rd week of the contact dermatitis induction) showing reduction of the lesion to small circumscribed area while the surrounding area appears normal. (d) GIII: After 2 weeks of treatment with pumpkin extract (end of the 3rd week of the contact dermatitis induction) showing marked improvement of the skin lesion apart from small areas with few scales

rats of GII showed ulceration and separation of the epidermis, while the superficial dermis shows marked inflammatory cell infiltration [Figure 2e and f]. Statistical analysis was done to compare epidermal thickness in the different studied groups using the *post hoc* test (least significant difference test). It was found that there was a significant increase ($P < 0.001$) in the epidermal thickness in GII compared to the control group (GI) [Figure 3a].

On the other hand, skin of rats of GIII treated with PE for 2 weeks after induction of CD with DNFB showed thinner epidermis, absence of focal elongation of the rete ridges, and reduced thickness of the granular layer. The dermis showed fewer inflammatory cells compared to those observed in GII [Figure 2g and h]. It was noticed that application of PE to the previously DNFB-painted skin areas resulted in a significant decrease ($P < 0.001$) in the epidermal thickness in GIII compared to GII [Figure 3a].

When the status and amount of collagen fibers in the skin dermis were assessed, skin sections were stained with Masson trichrome stain. It was found that skin of the control rats (GI) had intact collagen fibers in the dermis with regular loose arrangement of collagen fibers in the superficial dermal layer, while the deep dermal layer had irregular dense collagen fibers [Figure 4a]. Skin of rats of GII showed dense irregular collagen fibers in both superficial and deep dermis, while skin of some rats of the same group (GII) showed ulceration and separation of the epidermis as well as hemorrhage in the underlying dermis [Figure 4b-d]. When it came to the rats treated with PE after DNFB-induced CD (GIII), it was noticed that skin of these rats had regular less dense collagen fibers in the superficial dermal layer along with some inflammatory cells and red blood cells [Figure 4e-f].

The area percent of Masson-stained collagen fibers in the dorsal skin was compared in different groups using the *post hoc* test (least significant difference test). It was found that there was a significant increase ($P < 0.001$) in this area in GII compared to the control group (GI) [Figure 3b]. The area percent of Masson-stained collagen fibers in the dorsal skin significantly decreased ($P = 0.01$) in GIII compared to GII [Figure 3b].

Discussion

Skin irritation accounts for approximately 70% of all cases of CD. Chronic skin irritation results from repetitive exposure to chemical irritants. It often occurs in humans who perform repetitive wet work, and subsequently, is often a cause of occupational skin disease.[20] In two recent studies, pumpkin was reported to have a potential wound-healing effect. Bardaa

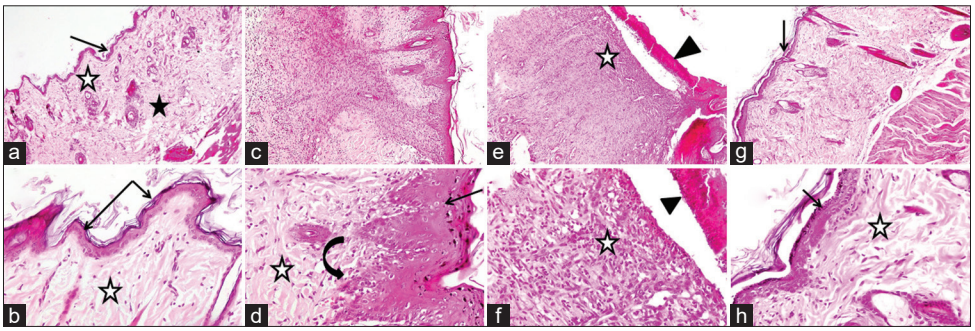

Figure 2: Sections of dorsal skin of albino rats stained with H and E stain. (a and b): GI: Control group showing thin epidermis (arrow), superficial dermis (white star), and deep dermis (black star). (c and d) GII: (1-fluoro-2,4-dinitrofluorobenzene-painted) showing hyperplastic-thickened epidermis with focal elongation of the rete ridges (curved arrow) and increased thickness of the granular layer (arrow).Inflammatory cells in the superficial dermis (white arrow) could be seen. (e and f) GII: (pumpkin extract-treated) showing ulceration and separation of the epidermis in some areas of the skin (arrowhead), while the superficial dermis shows marked inflammatory cell infiltrate (white star). (g and h) GIII: Showing thinner epidermis (arrow) and fewer inflammatory cells (white arrow) in the dermis compared to those seen in GII. (a, c, e, g, ×100, b, d, f, h, ×400)

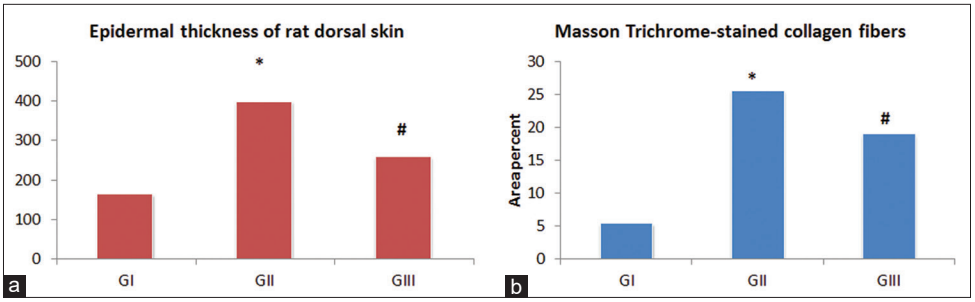

Figure 3: Epidermal thickness (a) and area percent of Masson's trichrome-stained collagen fibers (b) compared in the studied group using *F* test (one-way analysis of variance) and *post hoc* test with the least significant difference test. $P < 0.05$ was considered as statistically significant. *Significance versus GI, #significance versus GII

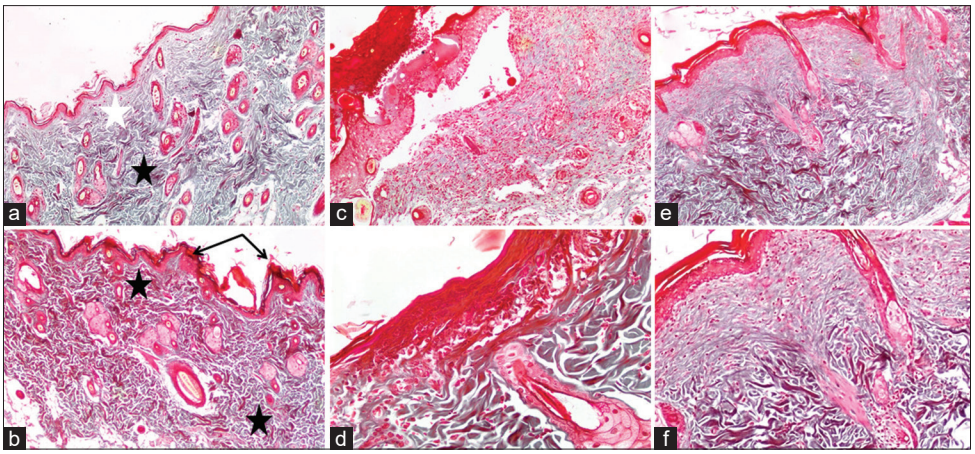

Figure 4: Masson's trichrome-stained sections of dorsal skin of the studied groups. (a) GI: Control group showing intact collagen fibers in the dermis note the regular loose arrangement of collagen fibers in the superficial dermal layer under the epidermis (white star) compared to the irregular dense arrangement in the deep dermal layer (black scar). (b) GII: (1-fluoro-2,4-dinitrofluorobenzene-painted) showing thickened epidermis (arrow) and dense irregular collagen fibers in both superficial and deep dermis (black star). (c and d) GII: (pumpkin extract-treated) showing ulceration and separation of the epidermis as well as hemorrhage in the underlying dermis. (e and f) GIII: Showing appearance of regular less dense collagen fibers in the superficial dermal layer along with some inflammatory cells and red blood cells. Note: The epidermis appears slightly thickened (a-c, e, ×100 f, ×200, d, ×400)

et al. proved that pumpkin oil has a promising effect on wounds healing in animal.[16] In addition, *C. moschata* peel extract was confirmed to have a wound healing activity on the second-degree burn in rats and could be a natural remedy for treatment of burns.[21] This is beside the reported antidepressant effect of sweet pumpkin.[15] Therefore, this study was designed to evaluate the efficacy of pumpkin fruit extract in treating CD in mice exposed to chronic stress.

Exposure to DNFB, in this study, induced CD in rats presented with dryness, hardness, and scaling, and this was associated with a significant reduction in the levels of antioxidants activity in the skin of rats. Microscopic examination of skin lesions showed hyperplastic thickened epidermis; superficial dermal inflammatory cells infiltrate and increased collagen fibers. Local administration of PE significantly increased the skin antioxidants activity and alleviated the CD-associated histopathological changes.

In this study, exposing rats to CUMS significantly increased the serum corticosterone which indicating the occurrence of chronic stress condition. This finding was previously reported in many studies and was attributed to stimulation of the hypothalamic–pituitary–adrenal axis.[17,22]

In this study, painting dorsal rat skin with DNFB for 2 weeks induced CD that was associated with a significant reduction in the skin antioxidants levels included SOD, GPX, and CAT. These results were in accordance those with previously reported by Kim *et al*.[15] It was said that CD have been associated with oxidative stress and inflammation and some skin irritant known to generate free radicals and reactive oxygen species (ROS).[23] The endogenous antioxidant defense system including the enzymatic antioxidants such as SOD, CAT, and GPx are overwhelmed by the extreme ROS generation that leads to a harmful oxidative effect in the skin, manifested by a marked decrease in the activities of SOD, CAT, and GPx.[24] This raises the issue that the appropriate approach to overcome these oxidative stress and inflammatory conditions is the use of antioxidants.

DNFB-induced CD in this study was macroscopically presented as dry, hard, and scaly skin. Among the histopathological changes observed were hyperplastic-thickened epidermis, infection and ulcerations in some areas and inflammatory cells infiltrate in the superficial dermis. Similar changes were reported in previous studies.[15,25] The harmful effect of benzene on the skin might be attributed to acting as organic solvents that may interrupt epidermal cell integrity, facilitating systemic absorption with subsequent toxic effects.[26] Welss *et al*. reported that solutions with lipid-solving properties induce skin irritation through damaging the hydrophobic barrier produced by the lamellar bodies producing layers or directly acting on keratinocytes leading to stimulation of the release of inflammatory cytokines such as interleukin-1α.[27]

Among the observations of this study was the presence of dense irregular collagen fibers in both superficial and deep dermis of rats suffered from DNFB-induced CD. In addition, there was a significant increase in the area percent of Masson-stained collagen fibers in the dorsal skin of rats of this group compared to the control group. These observations were in line with those previously described by Alshathly and Alqahtani 2017[28] in rats painted with benzene for 3 weeks.

PE was described to enhance antioxidant enzymes SOD and GPx activities and decrease lipid peroxidation in mice.[29] Bora *et al*. reported a significant reduction of *in vivo* antioxidant capacity of the skin following chronic exposure of experimental animals to broad-spectrum UV radiation.[30] They also observed a statistically significant increase in CAT, SOD, and GPx following the use of the formula of melatonin

and pumpkin seed oil. Moreover, this was evident in this study as the application of PE for 2 weeks resulted in a significant increase in skin activity of CAT, SOD, and GPx.

C. pepo (pumpkin) seed oil was reported to possess two classes of antioxidant compounds, namely; tocopherols and phenolics, which account for 59% of the antioxidant effects. It also contains L-ascorbic acid, which is the most important and abundant intracellular and extracellular aqueous-phase antioxidant.[31] *In vivo* antioxidant activity of pumpkin peel extract was described by Bahramsoltani *et al*., indicating the efficacy of antioxidant phytochemicals of the extract to adequately penetrate the damaged tissue.[21] Not only the pumpkin seed oil and peel that proved to have antioxidant power and radical scavenging activity but also pumpkin fruit extract was proved to have the same range of activity.[32] Xia *et al*. found that systemic application of PE reduced malonaldehyde (MDA) and elevated liver SOD and GPx.[33]

In this study, it was noticed that the administration of PE progressively decreased CD-associated dryness and scaling. It reduced the hyperplasic epidermis, the focal elongation of the rete ridges, and inflammatory cell infiltrate as well as increased collagen fibers. These effects might be attributed to the presence of flavonoids and phenolic compounds, which were described by Chonoko and Rufai when performed a phytochemical screening tests of *C. moschata* peel extracts.[34] These two compounds were reported to have anti-inflammatory and antioxidant effects.[34] Not only that, pumpkin was also reported to have an immunomodulatory activity evidenced by enhancing the proliferation of splenic lymphocytes and natural killer cells.[33]

Conclusions

Based on the aforementioned findings, it is postulated that the pumpkin fruit extract could have a potential in treating CD in stressed conditions mainly via its enhancement of skin antioxidant activity. According to the previous studies, pumpkin possesses a group of activities such as immunomodulatory effects that might count for its efficacy in treating CD that is planned to be investigated in future studies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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