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Anti-inflammatory and Anti-proliferative Activity of Coconut Oil against Adverse Effects of UVB on Skin of Albino Mice

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Abstract

Excessive exposure of ultraviolet type B (UVB) is the primary cause of skin issues like sunburn, swelling, hyperplasia, skin aging and cancer. Our objective was to assess the effectiveness of topical administration of refined coconut oil (RCO) and virgin coconut oil (VCO) against anti-inflammatory and anti-proliferative UVB adverse effects in mouse skin model by using of tumor necrosis factor-α (TNF)-α and transforming growth factor-β1 (TGF-β1). Twenty-four adult, *BALB/c* mice were allocated into 4 groups: group 1 (control negative) not exposed to UVB; group 2 (control positive) exposed to UVB only and left without treatment; group 3 exposed to UVB and treated by RCO topically; groups 4, exposed to UVB and treated topically by VCO. At day 35, the mice were sacrificed. Histomorphometry was achieved for their epidermal thickening with inflammatory reaction measurement, and the expression of TNF-α and TGF-β markers were estimated by immunohistochemistry. VCO reduced epidermal hyperplasia and thickness in comparison to the RCO group including; stratum spinosum thickness for the VCO showed (26.95±1.83μm) less mean than RCO group (39.88±4.24μm), stratum granulosum in VCO group (17.56±0.69μm) in comparison with the RCO (27.11±3.04μm). Regarding the stratum corneum thickness for the RCO (38.98±3.40μm) showed high mean than the VCO (21.49±2.08μm) and the inflammatory score was significantly decreased by (scores 2 and 1) for the RCO and the VCO, respectively as specified by the downregulation of TNF-α and TGF-β markers expression in the skin. The study concluded that anti-inflammatory and anti-proliferative effects of VCO contribute to antioxidant capacity.

Keywords: Albino mice, Anti-inflammatory, Anti-proliferative, TNF-α, TGF-β, Virgin coconut oil, UVB

1. Introduction

Skin, the biggest organ of the body, capacities as the fundamental boundary among the internal and the external milieu. Along these lines, it consistently shields the body from poisonous boosts, e.g., microorganisms, light (UV) illumination, allergens, aggravations and irritants (Lin *et al.*, 2017). Wavelengths in the UVB radiation (290-320 nm) of the sun oriented range are consumed by the skin and in charge of causing an increase in the epidermal, and to a lesser extent the dermal, mitotic activity, which persists from days to weeks, leading to an approximate two-fold thickening of the epidermis including acanthosis and parakeratosis, also thickening of dermis (Surget *et al.*, 2015). UVB also leads to physical inflammatory reactions, oxidative stress (Clydesdale *et al.*, 2001), immune suppression (Ullrich, 2005), DNA mutations, and ultimately non-melanoma skin cancer (Melnikova and Ananthaswamy, 2005).

UV light activates various flagging pathways that modify a translation. This procedure takes after the reaction to growth factors and is known as the UV reaction (Tyrrell, 1996). During UV radiation, the excessive formation of reactive oxygen species (ROS) can interrupt the stability among pro-oxidant production and antioxidant defense (Pillai *et al.*, 2005). ROS overproduction is an inducible factor brought about by the expression of cytokines, prostaglandins, leukotrienes and the pro-inflammatory molecules that elicit the arrival of inflammatory mediators to the site of disease that is broadly perceived by dermatitis (Lee *et al.*, 2003).

Cytokines such as tumor necrosis factor (TNF)-α plays an important role in photodamage and photoaging (Burke *et al.*, 2001). TNF-α released after UVB exposure induces endothelial cells and keratinocytes to display cell adhesion molecules, thereby recruiting inflammatory cells including keratinocytes, lymphocytes, macrophages and endothelial cells that secrete elastases and collagenases, leading to damage and aging of the skin (Rijken *et al.*, 2006; Moots *et al.*, 2018). TNF-α as pro-inflammatory cytokine also promotes apoptosis, lymphocyte activation, and hyperproliferative skin disorders (Banno *et al.*, 2004).

TGF-β is a family of pluropotent cytokines comprised of three isoforms in mammals such as TGF-β 1,-2, and-3, with TGF-β 1 predominant in most forms of tissue, including the skin (Li *et al.*, 2003). TGF-β 1 plays a critical role in sustaining homeostasis of the body by influencing cell development, differentiation, extracellular matrix accumulation, immune or inflammatory interactions and angiogenesis (Li *et al.*, 2003). UV irradiation induces TGF-β in both the epidermis and dermis of human skin. In the outer compartment of skin, TGF-β is a powerful negative regulator of keratinocyte proliferation (He *et al.*, 2002). Therefore, the induction of TGF-β by UV irradiation contributes to keratinocyte hyperplasia (Quan *et al.*, 2002) and also encourages the development of cancer (Massagué, 2012), TGFβ can encourage

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immunosuppression through immediate activation and modification of regulatory T cells (Tregs) (Sakaguchi and Powrie, 2007); also it effectively encourages expression of the fork-box protein P3 (Foxp3) in cluster differentiation (CD4 + T-cells) and converts it into a regulatory phenotype. (Chen *et al.*, 2003). Recent generation specific tiny molecule TGFβ pathway inhibitors, such as galunisertib, were shown to be safe, methods that reduce the length of therapy with TGFβ inhibitors may be preferred. (Neuzillet *et al.*, 2015).

Skin therapies emphasize combination therapy such as the use of moisturizers, antibiotics, antihistamines, and corticosteroids to treat skin inflammation to repair altered skin barrier function and reduce tingling. The use of steroids for immunosuppression and long-term topical application, however, decreases the amount of collagen causing skin atrophy (Oikarinen *et al.*, 1998). In impact, new therapeutic methodologies are being seriously studied due to these risks. Diverse plant species comprise a few bioactive components that have useful roles for health, such as antioxidant, anti-inflammatory, and antimicrobial effects, thus increasing their use for remedial purposes (Lin *et al.*, 2008). In animal models, several popular plant-derived products were tested for the development of anti-inflammatory therapies (Choi *et al.*, 2009). As a result, natural crop products are increasing as a new option for introducing certain diseases caused by free radicals in individuals, animals, food, and cosmetics (Lim *et al.*, 2007). The research found that vital oils are natural volatile compounds that exhibit powerful odors and are created by aromatic crops as secondary metabolites (Bakkali *et al.*, 2008).

Cocos nucifera fresh juice and kernel extracts, more commonly known as coconut, are usually used for their anti-inflammatory, antipyretic and wound healing characteristics in Southeast Asian countries (Zakaria *et al.*, 2011). Coconut oil is traditionally used for moisturizing and treating skin disorders. The emollient impact of coconut oil has been proved effectively in patients with atopic dermatitis, thus showing that coconut oil is a powerful natural soothing to be used in xerosis therapy (Verallo-Rowell *et al.*, 2008). Tocopherol and fatty acids (FAs) are important components of VCO and add to their antioxidant properties. These elements may cause sunburn, photoaging, and DNA degradation by cell protection (Marina *et al.*, 2009).

In this research, we intended to evaluate the effectiveness of topical administration of refined and extra virgin coconut oil against UVB negative impacts on the mouse's skin, and their effects on inflammation and cell proliferation were assessed using an inflammatory marker (TNF)-α and TGF-β1 proliferative marker.

2. Materials and Methods

2.1. Animal model and study design

In the animal house of the college of veterinary medicine, twenty-four adult albino mice (*Mus musculus* species, *BALB /c* strain) were purchased at a weight of 30-35 g. Mice were fed with standard pellet diet (Pico Lab) and provided with water *ad libitum*, were housed in the animal house/College of Veterinary Medicine/Sulaimani University, and were maintained at controlled room temperature about 25°C and photoperiodicity of 12 hours light/dark system. The animals were used according to the review and institutional guidelines of the Ethics Committee of the College of Veterinary Medicine/Sulaimani University (1235).

After 1 week of acclimatization, mice were allocated into four groups: Control negative group (n=6), mice were not irradiated to UVB irradiation and treated topically with phosphate buffer saline (PBS, 6 drops=300μL); Control positive group (n=6), mice were irradiated to UVB irradiation only and left without treatment (nor by PBS or coconut oil); Treatment group with refined coconut oil (n=6), mice were irradiated to UVB irradiation and treated topically with refined coconut oil (RCO) and the last group, which were treated with extra virgin coconut oil (n=6), mice were irradiated to UVB irradiation and treated topically with extra virgin coconut oil (VCO).

2.2. UVB irradiation

Our lamp phototherapy unit consisted of UVB lamps from the (Vilber-Lourmat-France), predominantly emitting UVB light at the range of 280-312 nm. The UVB fluencies used throughout the study were 80mj/Sec. With the exception of the control negative group, mice from other groups were exposed to UVB light for 35 minutes/day (4 days/week for 5 successive weeks) during the experiments. Electric shaver was used to cut the dorsal hairs for making a rectangular area (3*6 cm) prior to the UVB irradiation. Throughout the period of exposure, mice have moved around freely in a specially designed ventilated glass metal-free cabinet (32*25*25 cm).

2.3. Treatment of mice with coconut oils

Various kinds of coconut oils were used in this experiment, including virgin coconut oil (VCO) and refined coconut oils (RCO), both of which were purchased from (CalRoth, Germany). The analysis was carried out on each extracted type of coconut oil using high-performance liquid ultraviolet chromatography (HPLC-UV) separation on a silica column (Lichrosorb Si60 5 μm particle diameter, 250 mm length x4 mm id) in the Ministry of Agriculture (Baghdad, Iraq), and their chemical composition, including saturated triglyceride, was separated by the Marina et al method. (Marina *et al.*, 2009), whereas polyphenols, vitamins, and phenolic acid have been performed by Puah et al, protocol (Puah *et al.*, 2007).

The mice from both treatment groups were treated with refined and extra virgin coconut oils respectively 4 days/week. Oil treatments were performed topically in two different times 20 min before exposure to UVB (6 drops=300μL) and after UVB exposure directly (6 drops=300μL). During treatment, the quantity of both types of oil was evaluated by micropipette in order to regulate the amount of oil that was 300μL.

2.4. Tissue sampling and histopathological examination

The animals were anesthetized with ketamine and xylazine after 35 days of experimentation and then euthanized by cervical displacement; after that, skin samples were obtained from the dorsal skin, immediately fixed for 24-48 hours in 10 percent neutral buffered formalin and then passed skin samples for series histopathological preparations. Three transverse skin tissue segments (4 μm thick) were gained using a rotary microtome, hematoxylin, and eosin-stained first section,

IHC stained second and third section followed by microscopic assessment (Leica, Germany) and digital slide photography.

2.5. Histometric assessment

Dorsal skin slide sections were evaluated under a light microscope (Leica, Germany); equipped with an image analysis system (AmScope, AmView). In each case, the epidermal thickness was recorded in 3 layers after 35 days of the study, including stratum spinosum, stratum granulosum, and stratum corneum at 100 magnification (100x) independently, and then the mean was calculated for each layer in the group. When a picture was captured and then divided into 16 squares, inflammatory cells in the dermis were also counted, whereby all inflammatory cells (nuclear polymorphic cells and mononuclear cells) were counted at 400-fold magnification (400x), only the cells that included into the squares while those cells that located outside of it where excluded and mean numbers for each group were attained. Inflammatory cells were scored and categorized as follows: negative or score 0 (0-5 inflammatory cells), mild or score 1 (6-15 inflammatory cells), moderate or score 2 (16-25 inflammatory cells), and severe or score 3 (including 25 inflammatory cells).

2.6. Immunohistochemistry staining

Two skin sections (4 μm thick) were attached to the positively charged slides and allowed to dry in an incubator at 60 °C for 1 hour. With xylene and graded alcohol alternatives, the slices were deparaffinized and rehydrated. Antigen retrieval was accomplished by heating the sections in the pressure cooker that contained citrate buffer for 20 min. Through sinking the slides in 0.3 percent hydrogen peroxidase for 12 minutes, endogenous peroxidase activity was blocked. The sections were then coated with 3% (goat and mouse serum) to block non-specific bindings for about 30 minutes. The slides were then put in a damp chamber and incubated with rabbit anti-TNF-α polyclonal antibody (1:100, Biorybt, USA**,** orb7100) and rabbit anti- TGF-β1 polyclonal antibody (1:100, Biorybt, USA, orb11468) for 1 hr, followed by three washes in the buffer (2 min each). The sections were then incubated for 20 min with biotinylated anti-rabbit secondary antibodies (Biorybt, USA), washed in a buffer three times, incubated for 25 min in a Horseradish peroxidase-streptavidin (Envision, Biorybt) and washed in a buffer again four times. Tissue staining was visualized with the DAB substrate solution for less than 5 min (Biorybt, USA) and counterstained with hematoxylin. Then by a light microscope (Leica, Germany) examined the slides.

2.7. Assessment of immunohistochemical study

The result of immunohistochemical studies was analyzed quantitatively using the light microscope (Leica, Germany); equipped with an image analysis system (AmScope, AmView). The number of cells expressed TNF-α and TGF-β were calculated a magnification of 400x with in the same length of the epidermal layer for each section. TNF-α and TGF-β1 staining were scored and subsequently calculated in entire representative high power fields for each tissue sample. Three distinct observers (which were blinded to the experiment) subjectively assessed the magnitude and effect of the staining of each section using the following designations as in Table 1(Dong *et al.*, 2010; Jammal *et al.*, 2015).

Table 1. Immunoreaction scoring for the TNF-α and TGF-β1.

Quantification and intensity	Grades
No positive cell and no immunostaining	0
1-10% of positive cells and weak (light yellow)	1
11-25% of positive cells and moderate (yellow-brown)	2
26-50% of positive cells and focal strong (brown)	3
> 50% of positive cells and diffuse strong (brown)	4

2.8. Statistical analysis

A one way ANOVA and Duncan's test were used to assess the statistical significance between the groups. Statistical analysis was achieved using SPSS version 25.0 software (SPSS, Chicago, IL, USA). The results were presented as mean ± standard error (SE) and differences significant was reflected at $P< 0.05$; $P < 0.01$ and $P<0.001$.

3. Results

3.1. Chemical analysis of various coconut oil kinds

The analysis of various types of coconut oils was achieved by normal-phase high-performance liquid chromatography ultraviolet (HPLC-UV). In table 2, generally, the values for RCO were lower than extra VCO. However, fatty acid constituents were considered to be predominant and were about 87.98% in VCO while in the RCO were 78.18%. For example, lauric acid, which made up, the higher percentages among fatty acid (49.90%) in the VCO type in contrast to the RCO type that decreased to 44.89%. In general, less phenolic acids were detected in RCO (3.44%) compared with virgin oil (4.9%) samples because some phenolic compounds were lost or degraded during the refining process; Vanillic was the major phenolic acid in VCO ranged to 2.08% while in the RCO reduced to 1.80 only. Additional constituents that identified were polyphenols and vitamins, in the VCO made up 7.12% in comparison to the RCO type were dropped to 4.49%, and the Catechin was recognized as a significant component in both oil kinds, the unknown elements in the refined oil were 13.89%.

Table 2. Chemical components of various forms of coconut oils

	Components	Refined coconut oil %	Extra virgin coconut oil %
Saturated triglyceride (medium-chain)	Lauric acid	44.89	49.90
	Myristic acid	16.99	18.03
	Caprylic acids	6.80	8.10
	Palmitic acids	4.50	5.00
	Stearic acid	1.90	2.65
	Oleic acid	3.10	4.30
Phenolic acids	Protocatechuic	0.10	0.16
	Vanillic	1.80	2.08
	Syringic	0.03	0.45
	P-coumaric	0.45	0.12
	caffeic		
	Ferulic	1.06	2.09
Polyphenols and Vitamins	Gallic acid	1.05	2.0
	Catechin	1.60	2.90
	Vitamin E	0.95	1.02
	Vitamin K	0.89	1.20
Unidentified		13.89	

3.2. Histopathological finding

I- Control groups

Upon histologic examination, the skin section in control negative showed normal epidermal proliferation or thickness value that showed by mean error in each layer including standard error, stratum spinosum (7.86±1.28µm), in stratum granulosum (1.80±0.43µm), and stratum corneum (10.94±1.36µm) as revealed in table 3-5 and figures 1-3a, in comparison to the control positive group that showed significant increase in each mentioned layer's thickness. Histological changes such as diffuse epidermal hyperplasia or acanthosis, hypergranulosis, and hyperkeratosis were evident after 5 weeks of UVB irradiation. Acanthosis was noted in stratum spinosum with maximum mean (104.87±9.51µm) and highly significant value (*P=0.000*) as in table 3 and figure 1b. Also, severe hyperplasia of stratum granulosum or hypergranulosis was noted in which the granular cell layers increased or thickened to six or more layers from the normal one-three layers as in table 4 and figure 2b with a strong substantial value (71.20±10.26µm, *P*=0.00), additionally severe hyperplasia of stratum corneum (hyperkeratosis) also detected, in which the horny cell layer becomes abnormally thick and it measured (107.07±10.82µm) with significant value (*P*=0.000) as in table 5 and figure 3b.

II- Treatment groups

Histological analysis of skin sections in both treatment groups caused in a substantial reduction in UVB-mediated epidermal hyperplasia in contrast to the control positive group. In VCO epidermal hyperplasia mildly increased in contrast to the control negative group in each layer including stratum spinosum (26.95±1.83µm) with about 12.93 folds less than RCO group (39.88±4.24µm) with a substantial value (P=0.00) as in table 3 and figure 1c and d, that mean rose moderately, stratum granulosum in VCO group slightly increased their mean (16.52±0.79µm) in comparison with the RCO (27.16±3.03µm) that moderately thickened about 10.64 folds with significant value (*P*=0.00) as in table 4 and figure 2c and d. Regarding the stratum corneum also the RCO moderately thickened (38.98±3.40µm) about 17.49 folds with significant value of (*P*=0.02) in contrast to the VCO that mildly increased in their thickness (21.49±2.08µm) as in table 5 and figure 3c and d.

Table 3. Measurement (µm) of epidermal thickening (stratum spinosum) in different groups.

	Control negative (n=6)	Control positive (n=6)	Refined coconut oil (n=6)	Virgin coconut oil (n=6)
Mean±SE	7.03	81.64	30.25	28.00
	5.60	134.05	40.45	25.34
	6.26	112.0	38.01	30.40
	14.2	74.10	58.63	30.42
	7.10	122.97	41.54	18.64
	7.00	104.51	30.42	28.90
	7.86 ± 1.28^{a}	$104.87\pm9.51^{***b}$	$39.88\pm4.24^{**c}$	$26.95\pm1.83^{**d}$

Mean values with various small alphabetical superscripts differ from one another in the last row by $^{**}P<0.01$, $^{***}P<0.001$ vs. Control.

Table 4. Measurement (µm) of epidermal thickening (stratum granulosum) in diverse groups.

	Control negative (n=6)	Control positive (n=6)	Refined coconut oil (n=6)	Virgin coconut oil (n=6)
Mean±SE	2.00	65.03	39.28	15.21
	1.23	104.60	20.53	16.33
	1.80	47.05	23.8	15.98
	1.00	60.00	33.34	14.4
	3.8	50.12	22.10	20.00
	1.00	100.40	23.93	17.22
	1.80 ± 0.43^{a}	$71.20\pm10.26^{***b}$	$27.16\pm3.03^{**c}$	$16.52\pm0.79^{**d}$

Mean values with various small alphabetical superscripts differ from one another in the last row by $^{**}P<0.01$, $^{***}P<0.001$ vs. Control.

Table 5. Measurement (µm) of epidermal thickening (stratum corneum) in different groups.

	Control negative (n=6)	Control positive (n=6)	Refined coconut oil (n=6)	Virgin coconut oil (n=6)
Mean±SE	9.43	90.67	33.34	18.64
	7.25	100.96	32.34	30.69
	10.98	79.62	30.98	17.22
	8.34	91.60	50.93	23.93
	16.00	145.84	39.28	20.53
	13.67	133.75	47.05	17.98
	10.94 ± 1.36^{a}	$107.07\pm10.82^{***b}$	$38.98\pm3.40^{**c}$	$21.49\pm2.08^{**d}$

Mean values with various small alphabetical superscripts differ from one another in the last row by $^{**}P<0.05$, $^{***}P<0.001$ vs. Control.

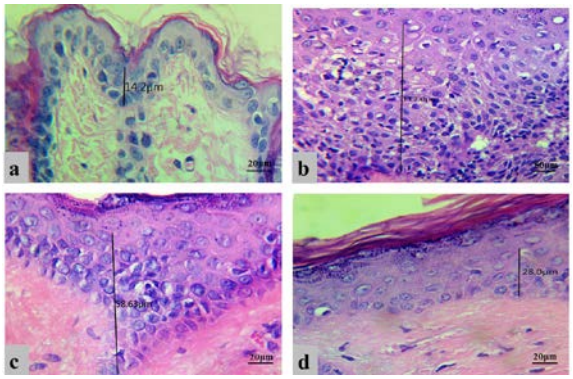

Figure 1. Histopathological skin sections of stratum spinosum thickness in mice. a: Normal thickening in control negative group, b: Marked acanthosis in control positive group, c: Focal-moderate thickening in RCO group, d: Focal-mild thickening in VCO group, (H and E stain).

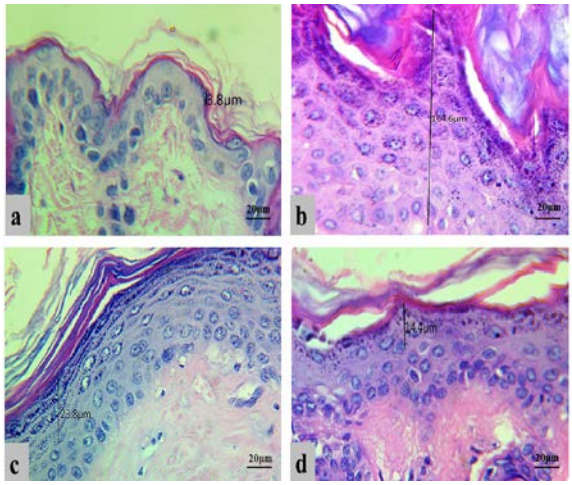

Figure 2. Histopathological skin sections of stratum granulosum thickness in mice. a: Normal thickening in control negative group, b: Severe hypergranulosis in control positive group, c: Moderate thickening in RCO group, d: Mild thickening in VCO group, (H and E stain).

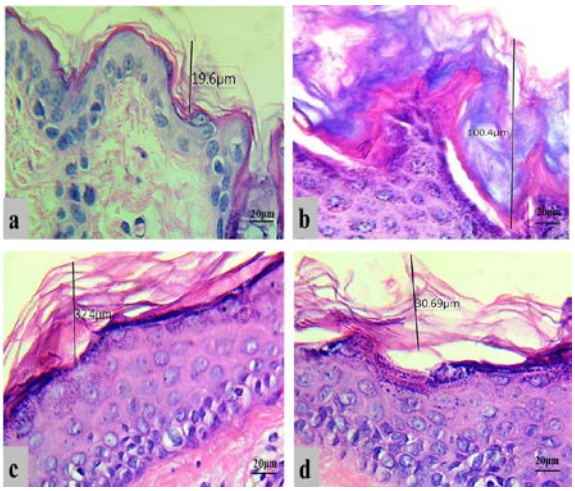

Figure 3. Histopathological skin sections of stratum corneum thickening in mice. a: Normal thickening in control negative group, b: Marked hyperkeratosis in control positive group, c: Moderate thickening in RCO group, d: Mild thickening in VCO group, (H and E stain).

3.3. Assessment of the inflammatory reaction

The statistical analysis of inflammatory polymorphonuclear and mononuclear was provided in Table 6. The UVB induced raising of inflammatory reactions (polymorphonuclear and mononuclear inflammatory cells) in control positive with highly significant value and the highest score was score 3 for all kinds of inflammatory cells with exception of neutrophil that showed significant only in contrast to the control negative group that showed score 0. In treatment group the infiltration of inflammatory cells was significantly decreased in the mean numbers and scores with scores 2 and 1 for the RCO and the VCO, respectively, in comparison to the control positive group, the differences were significant for all types of inflammatory cells, also among treatment groups, the inflammatory cells decreased significantly in the VCO as well as compared with the RCO with exception for the mast cells that showed no significant. The degree of inflammatory cells infiltration was showed in figure 4.

Table 6. The mean ± SE of the various groups of inflammatory cells.

Variables	Control negative	Control positive	Refined coconut oil	Virgin coconut oil
Neutrophil	1.00±0.60	2.83±0.63*	1.33±0.42	0.66±0.21*
Lymphocytes	1.33±0.55	7.33±1.08**	3.16±0.60*	1.00±0.25
Plasma cells	1.33±0.55	6.00±1.06**	2.66±0.66*	0.83±0.16*
Macrophages	1.50±0.50	8.66±1.70**	4.66±0.49**	4.16±0.47**
Mast cells	1.00±0.63	5.16±0.47**	0.83±0.30	1.16±0.30

Mean values with various small alphabetical superscripts differ from one another in the last row by *P<0.05, **P<0.01vs. Control.

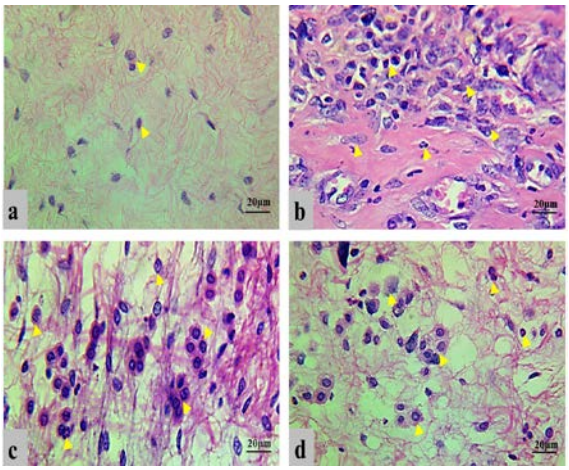

Figure 4. Histopathological skin sections of infiltration of inflammatory cells in mice. a: Normal infiltration in control negative group, b: Marked inflammatory reaction in control positive group, c: Moderate inflammatory reaction in RCO group, d: Mild infiltration in VCO group, (H and E stain).

3.4. TGF-β1 expression detection by IHC

Immunohistochemical analysis of the TGF-β1 marker shown in figure 5 that indicated the increasing of epidermal layer or proliferation of keratinocytes of epidermis, most common expression pattern found was cytoplasmic staining in the keratinocytes of the stratum spinosum of the epidermis of the skin, with a less TGF-β1 expression in the keratinocytes particularly in the stratum granulosum layer. Negative expression (Score 0) was seeming in mice of the control negative group, TGF-β1 expression was increased in the control positive group and showed strong-focal expression (Score 3) in mostly all keratinocytes of epidermis, when compared with the RCO

group, the TGF-β1 expressed in less than 25% of the epidermal keratinocytes which were stained moderate (Score 2). While in the VCO group the expression of TGF-β1 decreased if compared to the control positive and even to the RCO group that showed weak expression (Score 1).

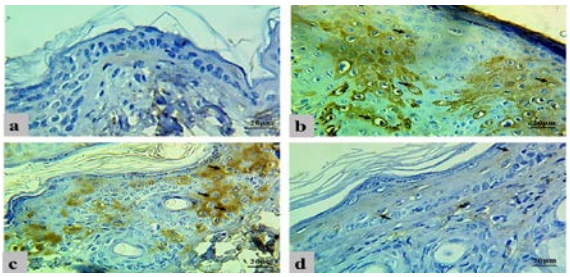

Figure 5. TGF-β1 expression in epidermal keratinocytes of skin section of different mice groups: a: Negative expression (Score 0) in control negative group, b: Strong-focal expression (Score 3) in control positive group, c: Moderate expression (Score 2) in RCO group, and d: Weak expression (Score 1) in VCO group, (Positive cells indicated by black arrows).

3.5. Immunohistochemical analysis of TNF-α

TNF-α expression showed in all groups with the exception of control negative, and its expression related to the increasing of inflammatory reaction in the dermis, immunohistochemical staining of the keratinocytes in mostly the stratum spinosum and less commonly in stratum granulosum of skin tissue sections that revealed variable scores of TNF-α expression and the pattern of expression was cytoplasmic. Immunohistochemical analysis of the TNF-α marker is shown in figure 6. Negative immunostaining was found in the control negative group (Score 0). In mice of control positive strong-focal expression (Score 3) was apparent, moderate expression of TNF-α (Score 2) was seen in mice of RCO group, whereas weak expression (Score 1) was seen in mice of VCO group in comparison to the control positive and RCO groups in which the TNF-α expression was decreased markedly.

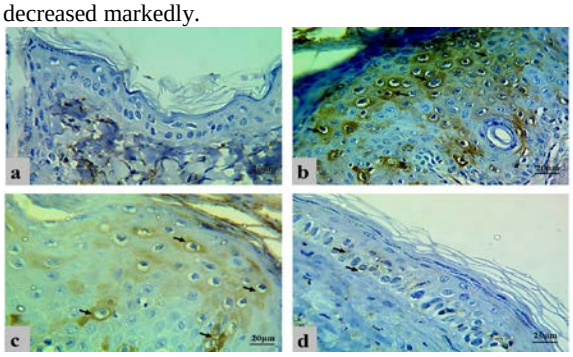

Figure 6. TNF-α expression in epidermal keratinocytes of skin section of different mice groups: a: Negative expression (Score 0) in control negative group, b: Strong-focal expression (Score 3) in control positive group, c: Moderate expression (Score 2) in RCO group, and d: Weak expression (Score 1) in VCO group, (Positive cells are indicated by black arrows).

4. Discussion

Plant oils have been discovered to have numerous helpful physiological features; therefore, they have long been used on the skin for cosmetic and medical reasons. For example, plant oil application can have an occlusive effect as a protective barrier on the skin, allowing the skin to retain moisture. Topical products also have the benefit of greater bioavailability in the skin and have a localized effect rather than systemic impacts (Patzelt *et al.*, 2012).

In this research, we examined the protective effect of RCO and VCO against UV exposure by following known markers of UV detriment (TNF-α and TGF-β). Our data demonstrated that topical coconut oils treatment on mouse skin significantly reduce epidermal hyperplasia that caused by UVB exposure particularly the group of mice that treated by VCO showed mild thickening of the stratum spinosum, granulosum, and corneum because VCO contain sufficient amounts of medium-chain fatty acids, polyphenols, tocopherols and free radical scavengers which not only improve the antioxidant status, but also reduce free radical-induced protein oxidative damage (Marina *et al.*, 2009; Arunima and Rajamohan, 2013), in contrast to the control positive group that showed marked hyperplasia of skin epidermis and in accordance with study who mentioned that epidermal thickening is regarded as the classical signs of UV exposure mediated damages to the skin (Reagan-Shaw *et al.*, 2006). Our information was also consistent with the earlier research, which documented that UV exposure causes oxidative stress in the skin that can be involved in a wide spectrum of circumstances, including hyperkeratosis and epidermal hyperplasia, which suggested influences on the mitotic cycle and on the macromolecular synthesis.

Triglycerides do not usually penetrate deeper into stratum corneum in plant oil; glycerol leads to the hydration of stratum corneum. Free fatty acids (FFAs), specifically monounsaturated FFAs such as oleic acid discovered in VCO rather than RCO, can interrupt the skin barrier and enhance the permeability of other compounds in coconut oils (Mack Correa *et al.*, 2014). This is the theory why our research showed mild hyperkeratosis to the group of mice who dealt with VCO. Other compounds found in different levels of each vegetable oil are phenolic compounds and tocopherols; they are the basic antioxidants found in virgin coconut oil. These compounds are very essential for the oxidative stability of purified fatty acid (PUFAs) in oil parts. For example, they have an effect on cancer prevention agents and can modulate physiological mechanisms such as homeostasis of the skin barrier, cell proliferation inhibition and inflammation (Servili *et al.*, 2009; Mack Correa *et al.*, 2014). This is the theory why the group topically treated with VCO diminished significantly epidermal hyperplasia instead of control positive group.

In our finding the group treated with RCO showed moderately epidermal thickening in contrast to the group treated with VCO that showed mild changes. The hypothesis why VCO had a potent effect than RCO is that the refined coconut oil is extracted from chemically lightened and deodorized coconut meat; therefore some of the components were decreased or altered by processing or by heating. While virgin coconut oil is obtained without the use of high temperatures or chemicals from the fruit of new, mature coconuts. It is regarded as unrefined and can provide health advantages because it contains elevated quantities of saturated triglycerides, phenolic acids, polyphenols and vitamins (Villarino *et al.*, 2007). Also, Marina et al. study revealed that VCO contained higher total phenolic content compared to refined coconut oil and, therefore, had more effective than RCO. It was suggested

that the RCO process being applied through the dry method had considerably destroyed some of the phenolic compounds in the coconut oil (Marina *et al*., 2009).

The UV-induced proliferation and epidermal hyperplasia depended on gene expression (Yamaguchi and Hearing, 2009). Our information also proved that the topical use of coconut oil also significantly inhibited UVB-mediated cell proliferation in addition to protein expression such as TGF-β1; for example, the RCO reduced TGF-β1 expression by score 2, while the VCO decreased TGF-β1 expression in score 1 in comparison to the control group that exhibited protein expression in high score (Score 3). This is an interesting observation because TGF-β1 expression has been revealed to be related with cellular proliferation and epidermal thickening that is regarded as a marker of proliferation after UVB irradiation for chronic duration in which in agreement with study of Ichihashi et al., who recognized that UVB generate ROS, and ROS have been shown to activate the latent form of TGF-β1 (Ichihashi *et al.*, 2003). Also, in agreement with former data, elevated TGF-β1 expression has been found in keratinocytes of the basal epidermal layer, and TGF-β1 overexpression associated with the acanthotic and hyperplastic epidermis suggesting that this factor is involved in a negative feedback growth regulatory loop (Li *et al.*, 1999; Liu *et al.*, 2001; Lu *et al.*, 2004). Our finding is in accordance with studies which verified that coconut oil is rich in medium-chain fatty acids such as lauric acid, capric acid, and myristic acid and have been documented to some extent, that such MCFA 's have anti-proliferative and apoptosis-inducing effects (Fauser *et al.*, 2011). These findings suggest that VCO can provide protection against UVB damage, and these protective impacts can be mediated through its antioxidant characteristics, which act as an anti-proliferative agent and reduce the expression of TGF-β1.

Due to UVB exposure, intracellular reactive oxygen species (ROS) play a crucial role in inflammation, aging, and cancer (Gause and Chauhan, 2016). Likewise, previous reports demonstrate that herbal oils have the capacity to defend against UVB irradiation. In this study, we have found that coconut oil more specifically the VCO markedly reduce the UVB exposure-mediated inflammatory reaction by score 1in comparison to the control positive group and the RCO also that have sore 3 and 2 respectively. In the present investigation, VCO demonstrated insurance against intracellular ROS delivered by UVB illumination irradiation. Our finding is in agreement with the previous report which described that the expression of the inflammatory profile was lower in the coconut oil-treated group after exposure to UVB radiation (Kim *et al.*, 2017). Topical coconut oil defends the cutaneous from UV irradiation (Korac and Khambholja).

For instance, leukocytes (PMNs, macrophages, and lymphocytes), mast cells, and dendritic cells are activated after an inflammatory response. Secreted cytokines such as IL-1α, TNF-α and-6 stimulate chemokines of chemotaxis that attract the immune cells to the injury and infection site and improve the inflammatory response (Reinke and Sorg, 2012). In the present study, the mice in control positive group showed strong expression of TNF-α (Score 3) with sever dermatitis by means of (29.98) because TNF-α is pro-inflammatory cytokine and is significant initiator cytokine of inflammatory responses (Neurath *et al*., 1997).

Our study is in accordance to the former study which mentioned that epidermal keratinocytes, react to proinflammatory cytokines like TNF-α by involving in the expression of many inflammatory mediators during the chronic inflammatory skin disorders induced by UVB irradiation. The chemokines and growth factors of these keratinocytes are the primary force behind the accumulation and proliferation of inflammatory cells in the skin, thus preserving chronic inflammation of the skin (Pastore *et al.*, 2005). This study confirmed that VCO was potent in reducing the TNF-α expression (Score 1) and inflammatory reaction (mild inflammation) in dermis by mean of 7.81 and the inflammation was decreased by 22.17 folds in contrast to the control positive group and RCO group that showed moderate expression of TNF-α (Score 2) and moderate dermatitis by means of 12.64, when compared to the control positive group the inflammatory reaction dropped by 17.34 folds. Our results agree with the study of Varma et al., 2019, who proved that topical use of VCO inhibits anti-inflammatory activity by inhibiting different concentrations of cytokine including TNF-a, IFNg, IL-6, IL-5 and IL-8 and recovers skin hyperplasia and inflammation (Varma *et al.*, 2019). Other studies documented the anti-inflammatory effects of VCO in reducing atopic dermatitis and enhancing moisturizing to the skin (Evangelista *et al.*, 2014). The outcome suggests photoprotective and anti-inflammatory impacts of VCO against UVB irradiation, making it a significant ingredient in formulations and warranting further clinical studies owing to elevated levels of phenolic and antioxidant elements.

5. Conclusion

From the two types of coconut oil obtainable, VCO appears to carry the highest potential and is more beneficial for skin health by reducing epidermal thickening and inflammatory reaction because its composition varies and contains a higher amount of saturated free fatty acid, polyphenols, and vitamin E compared with RCO. The research indicated that VCO had anti-inflammatory and anti-proliferative impacts through its antioxidant characteristics.

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