



## Photo Protective Role of Wild Edible Plants on Skin of Mice from Harmful Effects of Ultraviolet Type-B Irradiation

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### Authors' contributions

*This work was carried out in cooperation between all authors. Author SMAH designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. All authors managed the analyses of the study and literature searches. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aim:** The aim of this study was to determine the protective antioxidant capacity of edible wild plants in the decreasing effect of ultraviolet B (UVB) on the skin of albino mice.

**Study Design:** Thirty skin biopsies were taken from the mice to detect and compare the hyperplastic effects between the groups.

**Place and Duration of Study:** Animal House of College of Veterinary Medicine, Sulaimani University, Histopathology Lab of Consultative Hospital/Sulaimani Governorate, Anatomy and Histopathology Department, College of Veterinary Medicine, Sulaimani University.

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**Methodology:** Thirty mice (*Mus musculus* species, BALB/c strain) underwent this study and were divided into six groups from A-F, according to the UVB exposure, species of plant, and routes of administration. Mice from all groups (Exposure and plant administration except control group) were subjected to UVB irradiation four days/week (20 minutes/day, four weeks), and treated with edible wild plants (*Gundelia tournefortii* and *Malva sylvestris*) by different routes.

**Results:** According to the Student's t-test (Paired) and Pearson's correlation coefficients we found that the effect of *Gundelia tournefortii* and *Malva sylvestris* were highly significant in reducing the hyperplastic effect of UVB irradiation.

**Conclusion:** This study gives an overview of traditional uses of *Gundelia tournefortii* and *Malva sylvestris* against the adverse effects of UVB radiation.

**Keywords:** Ultraviolet type-B; *Malva sylvestris* L; *Gundelia tournefortii* L; antioxidant; anti-inflammatory; epidermal thickness.

## 1. INTRODUCTION

Reactive oxygen species can be generated by daily exposure to ultraviolet light and may cause some sub chronic and chronic disorders [1]. It has been known for decades that reactive oxygen species (ROS) produced in the body following UV irradiation are key mediators of oxidative damage. Cell damage from UV also occurs through many mechanisms that include: peroxidation of membrane lipids via generation of lipid peroxides, rapid depletion of several endogenous enzymes and antioxidants such as glutathione reductase and catalase [2]. Exposure to UV has also been shown to induce pro-inflammatory cytokines, such as IL-1 $\alpha$ , and matrix metalloproteinase (MMPs) in skin cells such as keratinocytes and fibroblasts [3].

The human and animal body have multiple mechanisms and antioxidant systems that protect the cellular molecules against damage induced by free radical [4]. In fact, the balance between free radicals production and antioxidants is thought to be strongly related to lifespan [4]. However, these systems don't exercise sufficient protection against oxidative stress. Most of the exogenous antioxidants are constantly required to maintain an adequate level of antioxidant so that the ROS in the human and animal body are balanced [5]. Antioxidants are usually classified into two groups, specifically, synthetics and natural, in addition, numerous synthetic antioxidants have been developed. The phenolic compounds such as vitamin E, flavonoids, tocopherols, carotenoids as well as ascorbic acids are typical antioxidants that have been used [6,7]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) have been used since the beginning of this century. The diet rich in polyphenolic compounds and flavonoids is

associated with longer life expectancy because according to the most research discovery, antioxidant compounds have properties such as anticancer, antiviral, anti-inflammatory activities [8].

Nowadays, there has been an increasing interest in consuming wild food plants [9,10]. In the face of the agricultural society's primary dependence in collecting plants, the tradition of eating wild plants has not completely disappeared and their nutritional role and health benefits are being reported in many surveys world-wide [11-13].

*Malva sylvestris* belongs to the mallow genus in the family Malvaceae mortician. It is commonly known as mallow in Europe, gulkhaira or vilayatti kangani in Pakistan and India [14,15], recently *Malva* is well-known using plants because many researchers are focusing on its therapeutic properties, such as, antioxidant, anti-inflammatory, anticancer, and, attenuation of skin disorder symptoms [16,17].

The *Gundelia* is a spiny, thistle-like flowering plant of the genus *Gundelia* L. In the sunflower family named by Asteraceae [18,19]. *Gundelia* is common name of *G. tourenfortii*, and tumbleweed, akub (kuub or aqub), respectively are traditional English and Arabic equivalents for this plant. *G. tourenfortii*, locally known as "kangar" in Kurdistan, is found as a wild herb growing during late winter and early spring [20-22]. *Gundelia* revealed to have several pharmacological effects, e.g. Antibacterial, anti-inflammatory, hepatoprotective, anti-spasmodic, antiseptic, antioxidant [23,24]. *Gundelia tournefortii* is a medicinal plant of *Gundelia* a genus that is used for the treatment of pain, inflammations, liver disorders, stomach ache, diarrhea, bronchitis, nephrolithiasis [25,26].

The aim of this study was to determine the antioxidant protective role of *Gundelia tournefortii* and *Malva sylvestris* in decreasing the damaging effect of UVB on the skin of albino mice.

## 2. MATERIALS AND METHODS

### 2.1 Animal Model

Adult albino *Mus musculus species*, BALB/c strain (15 males and 15 females) was used in this experiment, which was fed with standard pellet diet (Pico Lab) and provided with water and *ad libitum*. Animals were housed in the animal house of College of Veterinary Medicine, Sulaimani University under a controlled room temperature of about 25°C and photo-periodicity of 12 hours light/dark system. Animals were assigned into six groups: Group A (Control group, n=5) which were not exposed to UVB and not administrated with plants, Group B (n=5) exposed to UVB irradiation only, Group C (n=5) which were exposed to UVB light and administrated with *Malva sylvestris* orally before UVB irradiation, Group D (n=5) which were irradiated with UVB light and painted with *Malva sylvestris* before UVB irradiation, group E (n=5) which were exposed to UVB light and administrated orally with *Gundelia tournefortii* before UVB irradiation, and Group F (n=5) which were irradiated with UVB light and painted with *Gundelia tournefortii* before UVB irradiation. All Groups except control one were exposed to UVB for about 20 minutes, four days/week for one month consecutively.

### 2.2 Collection of Plants

*Malva sylvestris* and *Gundelia tournefortii* were collected from the local market of Sulaimani/Kurdistan Regional Governorate (KRG).

#### 2.2.1 Preparation method and administration of plant materials

##### 2.2.1.1 *Malva sylvestris*

*Malva sylvestris*, which was distributed throughout most villages of Sulaimani/KRG, which was named by toleke (Fig. 1a). Fresh *Malva sylvestris* (whole plant) was collected from the market and washing many times by plenty water, The 3 kg fresh plant has been boiled in a

container that contains 1 L of tap water for about 10 minutes, then the fluid or juice of plants were filtered and their stored in refrigerator in 8°C till the time of its using, each mouse from group C were administrated orally with 1ml of *Malva sylvestris* before UVB irradiation and also each mouse from group D were painted with *Malva sylvestris* by cotton for about 5 minutes before UVB irradiation throughout the experimental study.

##### 2.2.1.2 *Gundelia tournefortii*

*Gundelia tournefortii* L., Which was named by Kangar (Fig. 1b), were scattered throughout most villages of Sulaimani/ KRG. Fresh (whole plant) were collected and washed, the 3 kg of this plant mixed with 1 L of water and boiled for about 15 minutes, then it was filtered and their juice stored in 8°C degree of temperature till the time of its using, each mouse from group E were administrated orally with 1 ml (3 mg/1 mL) of *Gundelia tournefortii*, before UVB exposing and mice from group F were painted with *Gundelia tournefortii* by cotton for about 5 minutes before UVB irradiation throughout the experimental study.

### 2.3 UVB Lamp

The Lamp that was used in this experiment was about 312 nm wavelength, 15 Watts, VILBER-LOURMAT-FRANCE, with a calculated power 80 mj/ Sec. with exception of control group mice from all groups were exposed to UVB light for 20 minutes 4 days/week (4 weeks) consecutively, and this was done after shaving the mouse's back skin (2x5 cm).

### 2.4 Sampling Method

At the end of the experiments, the animals were euthanized using (Xylazine-Ketamine: 0.1 mL/10 gm of body weight) as recommended dose intraperitoneally and cervical dislocation. Tissue samples were taken from skin tissue. The tissues specimens were fixed in 10% neutral buffered formalin for at least 24 hours and then routinely processed that were performed in the Histopathology Lab of Consultative Hospital/Sulaimani Governorate. The samples were paraffin embedded and a section of 5µm thickness to detect and compare any abnormal lesions formed by UVB and in administrating plants' groups in skin tissue.



Fig. 1. a, b. *Malva sylvestris* species; c, d. *Gundelia tournefortii* species

## 2.5 Morphometric Study

The epidermal thickness was measured in Haematoxylin and Eosin-stained sections at a magnification of 100 using the image analyzer (Scope image software 9.0 "H3D" computer system-England, digital binocular compound microscope), in the Anatomy and Histopathology Department, College of Veterinary Medicine, Sulaimani University. Five different fields of the epidermis were randomly chosen from each section. Epidermal thickness was defined as the distance between the basement membrane and the apical surface of the uppermost layer (from stratum basale to the top layer stratum corneum).

## 2.6 Statistical Analysis

For each case, the epidermal thickness was measured in five high power fields by, then mean was obtained and at the end the measurements of each epidermal skin thickness (Mean  $\pm$  S. E. M) of different groups were compared with a corresponding epidermal skin thickness of the control group and between each group. The Student's t-test (Paired) and Pearson's correlation coefficients were used to estimate the skin thickness of the back region and in all analyzed, a  $P = 0.05$  was considered to be significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Gross

Gross examination was done by palpation back skin for estimation of thickness in all groups and

visual inspection of any lesions that developed in the backs' skin as shown in Fig. 2. In group A, skin showed delicate and thin appearance (Fig. 2a). While in the group B different lesions were observed due to UVB exposure that ranged from erythema, edema, ulcer of the exposed area and epidermal thickness as shown in (Fig. 2b), in comparing to group B the skin thickness of the groups; C, D, E and F developed a variable epidermal thickness that detected during palpation as in (Fig. 2c-f). Our result was by the result that proved oxidative damage of UVB that contributes to a variety of skin illnesses, including inflammation, degenerative aging, hyperplasia and cancer [27]

### 3.2 Light Microscopic

The results of this study have demonstrated different histopathological changes that are shown in Fig. 3. Examination of sections stained with H&E from the control animals (Group A) revealed the normal histological structure of the epidermis and dermis layers of the thin skin. The epidermis was composed of stratified squamous epithelium, formed mainly by keratinocytes that appeared to be arranged in 2-3 layers. The border between the epidermis and dermis was clearly demarcated. The underlying papillary layer of the dermis had abundant capillaries and connective tissue cells, whereas the inner reticular layer of the dermis was composed of a denser connective tissue rich in fiber as shown in (Fig. 3a). In group B, there was a highly epidermal proliferation or hyperplastic epidermis in a responded to UVB exposure as in (Fig. 3b), however, in group C and D (Fig. 3c,d), there was



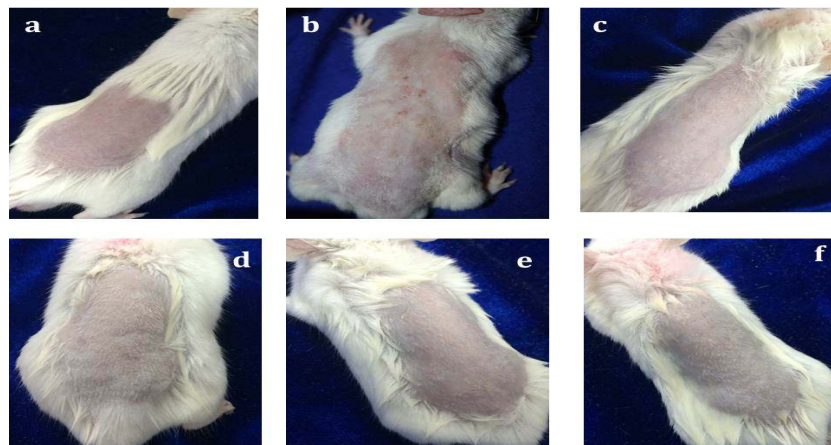
a mild-moderate epidermal hyperplasia due to administration of *Malva sylvestris* orally and painted routes, in the group E and F developed normal-mild epidermal hyperplasia, due to treatment by *Gundelia tournefortii* orally and painted routes (Fig. 3e, f).

The observed lesion that found in the dermis was shown diffusely infiltration of the inflammatory cells including neutrophils, macrophages, and lymphocytes in the dermal layer of group B.

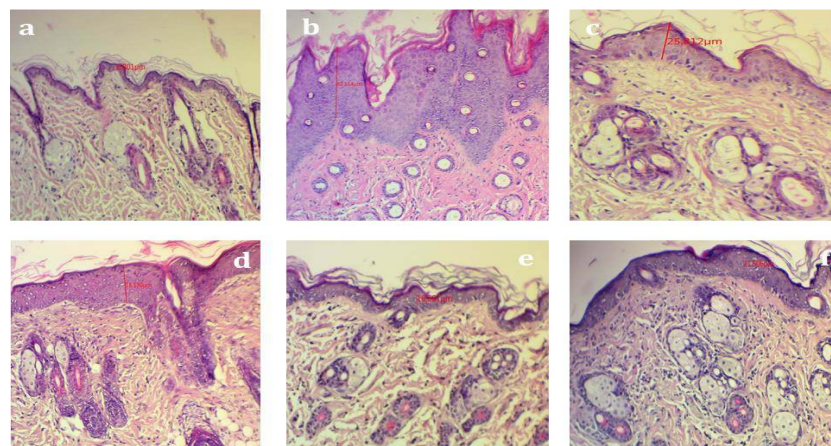
While in the group C and D, there was mild-moderate infiltration of inflammatory cells when

comparing to group E and F that show normal-mild infiltration of inflammatory cells as shown in (Fig. 4a-f).

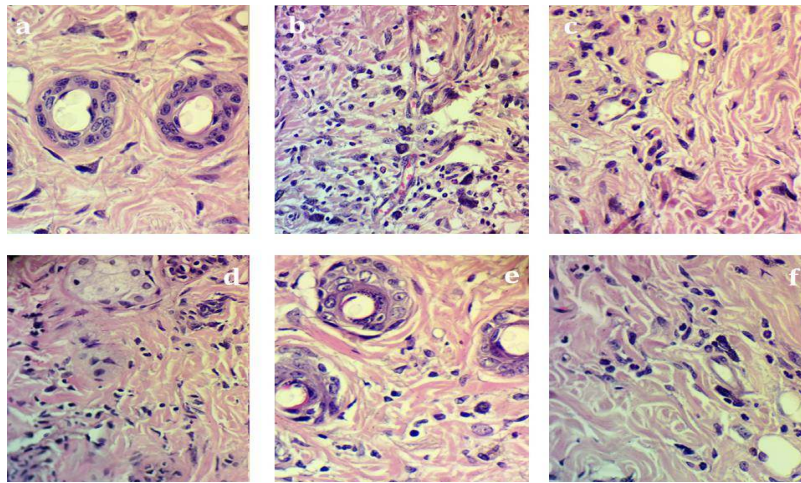
This study reported an extremely significant association between UVB irradiation and increasing epidermal thickness as we detected epidermal thickness in exposure group 12.93 times greater than the control epidermal thickness, this finding agrees with previous studies [28,29], which demonstrated that UVB radiation on the skin induces a variety of changes in the epidermis, including keratinocyte proliferation that leads to epidermal thickness.



**Fig. 2. a. Normal skin thickness in the control group; b. Erythema, ulceration and severely increasing of skin thickness in exposure group; c,d. Slight or mild-moderate increasing skin thickness in orally administered and painted with *Malva sylvestris* groups; e,f. Normal-slight skin, increasing thickness in orally administered and painted with *Gundelia tournefortii* group**



**Fig. 3. a. Normal histologically of epidermal thickness in the control group; b. Diffuse epidermal proliferation in exposure group; c,d. Mild-Moderate epidermal proliferation in orally administered and painted with *Malva sylvestris* group; e,f. Normal-mild epidermal proliferation in orally administered and painted with *Gundelia tournefortii* group (X100, H&E stains)**



**Fig. 4. Microscopic appearance of dermal infiltration shows: a. the dermis of control mice, which contains the normal cell constituent; b. Diffuse infiltration of inflammatory cells in the exposed group; c,d. Mildly-moderate infiltration of inflammatory cells in orally administered and painted with *Malva sylvestris* group; e,f. Normal-mildly infiltration of inflammatory cells in orally administered and painted with *Gundelia tournefortii* group (X400, H&E stains)**

Another useful role of *Gundelia tournefortii* and *Malva sylvestris* administration, despite the reduction of the epidermal thickness effect of UVB in this present study is their role in decreasing the inflammatory cell infiltration, this finding is in agreement with the previous study, which reported the role of both edible plants as an anti-inflammatory agent, and specially the *Gundelia tournefortii* species that have shown an extremely significant effect in decreased inflammatory cells [30-32]. The present study was first to display the role of both edible plants on reducing inflammatory cells in mice by chronic UVB irradiation.

### 3.3 Morphometric Analysis

As assessed by the image analyzer. The epidermal measurements for the cases and control groups were presented in Table 1. The control group was measured as  $7.065 \pm 0.129$ . There was a highly significant increase in the mean thickness of the epidermis in group B ( $t = -17.052$ ,  $P = 0.000$ ) and their mean was  $(91.416 \pm 4.825)$ , when compared with the control group and its' full epidermal thickness was 12.93 greater than the control group, the mean thickness of the epidermis in group C was  $(26.856 \pm 3.247)$ , when compared with the control group mildly increased ( $t = 6.223$ ,  $P = 0.003$ ), where their epidermal thickness was only 3.75 times increased, however comparing to group B its highly significant for using this plant as antioxidant

agent ( $t = -8.166$ ,  $P = 0.001$ ) and their thickness or proliferation was mildly increased. In group D ( $29.761 \pm 1.716$ ), their thickness was moderately increased when compared to control group ( $t = 13.707$ ,  $P = 0.000$ ) and its epidermal measurement difference to group B was ( $t = 8.166$ ,  $P = 0.001$ ), where full epidermal thickness was 4.09 than group B, and it showed highly significant that decreased the role of UVB about 5.09 times. In both groups of E and F especially group E epidermal proliferation is close to normal when comparing two groups A and B, for group E ( $18.978 \pm 2.069$ ), ( $t = 10.835$ ,  $P = 0.000$ ), only 2.68 times increased than group A and B, whereas in group F ( $27.029 \pm 1.835$ ), ( $t = 9.640$ ,  $P = 0.001$ ), while their epidermal thickness time was about 3.82 fold increased it meant that *Gundelia tournefortii*, 6.43 times reduced the hyperplastic role of UVB in comparison to exposure group.

In this current study, we provided protection against the UVB-induced epidermal thickness by oral and painted administration of *Malva sylvestris* and *Gundelia tournefortii* species. Due to the biological activity of these plants that may be attributed to antioxidants, such as polyphenols, vitamin C, vitamin E, carotene, and other important phytochemicals, which protect skin against UVB irradiation [33]. The present finding showed that protective effect of *Gundelia tournefortii* species was highly significant in reducing the hyperplastic effect of UVB than the

*Malva sylvestris* The result of this finding agrees with the publications of [34], who reported that free radical scavenging activity values of *Gundelia tournefortii* that includes Total phenol, flavonoid, alkaloid and ascorbic acid exhibited stronger value than those found in *Malva sylvestris* species. The similar result [35-37], which reported that the total phenolic content and antioxidant activity in this plant are attributed to Gallic Acid and Quercetin with typical flavonoids that include; Kaempferol and Quercetin. These antioxidant components inhibit glutathione-S-transferase activity and suppress the generation of reactive oxygen species.

In this study, we proved the effect of *Gundelia tournefortii* Species orally was more effective than painted one and showed highly significant by reducing the epidermal thickness to 3.82 times, therefore using this edible plant has a powerful value in protecting skin from harmful effect of UVB. Our study in agreement with studies [38-41], who proved the oral administration of *Gundelia tournefortii* is useful in treating several disorders including; liver diseases, diabetes, chest pain, heart stroke, gastric pain, vitiligo, diarrhea, and bronchitis. It is also reported to have hypoglycemic, anti-inflammatory, anti-parasite and antiseptic. To my knowledge, no study has been reported the

antioxidant effect of this plant in reducing the role of UVB exposure.

Our results it found using of *Malva sylvestris* was effective in reducing UVB-inducing epidermal thickness development, it revealed that the oral administration showed highly significant association that was 4.09 time, when compared to UVB group and this demonstration proved that this plant is an excellent source of antioxidant against oxidative stress of UVB, and this result is in agreement with the publication of [42], who showed that Kaempferol-3-Orutinoside and quercetin-3-O-rutinoside were the main flavonoid component in this edible plant, this finding is inconsistent with the previous studies, who reported that oral administration of *Malva sylvestris* has lowered highly oxidizing reactive oxygen species, due to presence of flavonoids, phenolic component that usually involves several antioxidant potential activities [43,44]. The results of this study were the first of its kind to be reported.

Epidermal thickness according to Pearson correlation coefficient test as shown in Table 2. In group B epidermal thickness was increased in comparison to the group A, and shown a negative, high correlation between them (r=-0.936, p=0.000), also, a negative, strong correlation was found between the group C, D

**Table 1. Epidermal thickness measurement (µm) for different individual cases in groups**

Groups Cases	Group A control	Group B exposure	Group C Malva –Oral	Group D Malva- Painting	Group E Gundelia- Oral	Group F Gundelia- Painting
1	6.685	101.9	18.035	27.383	11.597	21.506
2	6.901	99.7	22.804	25.627	18.507	25.348
3	7.1	91.4	25.812	28.004	19.609	27.074
4	7.22	82.514	31.325	31.623	21.095	28.57
5	7.422	81.566	36.308	36.17	24.083	32.653
Mean±SEM	7.065±0.129	91.416±4.825	26.856±3.247	29.761±1.716	18.978±2.069	27.032±1.835

**Table 2. The correlations between different groups by using Pearson’s correlation coefficient test**

Groups	Group A	Group B	Group C	Group D	Group E	Group F
Group A	1	-0.936(*)	0.963(**)	0.933(*)	0.974(**)	0.992(**)
Group B		1	-0.937(*)	-0.952(*)	-0.858	-0.957(*)
Group C			1	0.976(**)	0.915(*)	0.969(**)
Group D				1	0.840	0.932(*)
Group E					1	0.969(**)
Group F						1

\*\* Correlation is significant at the 0.01 level (2-tailed)

and group B ( $r=0. -0.937$ ,  $r=-0.952=P=0. 000$ ), that showed its protective effect on UVB radiation. In group E and F also a negative, strong correlation was found ( $r=-0.858$ ,  $r= -0.957$ ,  $P=0.000$ ) when compared to Group B that was more efficient than group C and D, there was a strong, positive correlation that also found between individual groups

#### 4. CONCLUSION

The result obtained in the present study indicates that these edible wild plants it considered as a protective valuable traditional using plants against the full range effects of UVB radiation.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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