RESEARCH PAPER

Chemical Composition of Grape Seed Extract by GC-MS and Antioxidant Potential and Regenerative effect in Electrospun Nanofibers for UV. Induce Skin Damage

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ABSTRACT

Solar radiation is essential for the continuation of life on earth, although it have many benefits to living organisms the quantity and quality of ultraviolet radiation reaching the earth's surface can give rise several histological changes in domestic animals. The bioactive composition in grape seeds incorporate into Electro spun nanofibers scaffold exhibit strong antioxidant activity. The recent technology of electrospinning nanofibers ameliorates the ability of biomimetic scaffold of delivering bioactive compound for tissue remodeling and re-architecture which may reduce the histological damage rabbit skin from UV. Oxidative stress. Evaluate the antioxidant efficacy of grape seed extract incorporate Electro spun nanofibers and ability to regeneration and re-architecture of skin tissue in exposure rabbit to ultraviolet radiation.

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INTRODUCTION

Ultraviolet radiation effects the skin in a variety of ways, some pf which are immediate like skin burn and others which take time to emerge as aging, and DNA damage, and the progression of skin carcinogenesis. long-term exposure to solar radiation lead exaggerated production of reactive oxygen species (ROS) resulting in cellular oxidative stress, oxidation cell membrane lipid, protein degeneration and hyper proliferation of keratinocytes and DNA damage.[1]

Prolong UV. exposure is characterized hyperkeratosis multiple projection of epidermis fluid accumulation between keratinocytes of epidermis resemble a sponge and fragmentation and disarrangement of elastic fibers, these changes lead to structural and functional fragility of the skin barrier and premature skin aging and increased risk of skin carcinogenesis, as proven by numerous studies related to this biological aspect [2-4].

Recently many studies focused on fabrication technique creates nanofibers that can be mix with various materials as well as capability to resemble the extracellular matrix, providing an optimal environment for skin tissue regeneration [5]. Generally, nanofibers create through the electrospinning technology have potential

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database in distribution and delivery of drug therapies in addition reinforce the ability of cell to heal and tissue regeneration [8,9]. Grape seeds have garnered significant attention from numerous studies and researchers as a natural therapeutic agent due to their ability to neutralize reactive oxygen species, reduce oxidative stress, and repair damage caused by prolonged ultraviolet radiation. Therefore, the bioactive compounds in grape seeds are being identified through the analysis of volatile and semi-volatile phytochemical (GC-MS) to provide valuable information on the active chemical composition of grape seeds and to link these compounds to their therapeutic efficacy and ability to neutralize free radicals. this study aimed to evaluate effectiveness of bioactive compound in electro spun grape seeds extraction loaded on medical polymer PVA/CS [10-12], and nanofibers scaffold ability to repairing damage tissue from prolong UV. exposure [13,14]. Scanning electron microscopy (SEM) has become an advanced tool for observing the three-dimensional ultrastructural properties of biological tissues, enabling in-depth observation of surface morphology and spatial relationships, which conventional light and transmission electron microscopy are not capable of observing [15].

MATERIALS AND METHODS

GC–MS Analysis

The chemical composition of grape seed extract was analyzed using Gas Chromatography–Mass Spectrometry (GC–MS) to identify its bioactive constituents. This technique is widely used for the qualitative and quantitative analysis of volatile and semi-volatile compounds. The analysis was carried out at the Central Laboratory, College of Agriculture, University of Basrah, Iraq.

For sample preparation, 5 g of dried grape seed powder was extracted with 50 mL of HPLC-grade methanol using a Soxhlet apparatus for 6 h. The extract was filtered through Whatman No. 1 filter paper and concentrated at 40 °C under reduced pressure using a rotary evaporator (Buchi R-210, Switzerland). The concentrated extract was then dissolved in 5 mL of methanol and passed through a 0.22 µm PTFE syringe filter before GC–MS analysis.

The analysis was performed using an Agilent 7010B Triple Quadrupole Mass Spectrometer (Agilent Technologies, USA) coupled with a Finnigan Focus Gas Chromatograph (Thermo Scientific, USA). Separation of the compounds was achieved on a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was initially set at 60 °C for 2 min, then increased to 280 °C at a rate of 5 °C/min and held at this temperature for 10 min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector and detector temperatures were maintained at 250 °C and 280 °C, respectively. Mass spectra were obtained using electron impact (EI) ionization at 70 eV over a mass range of *m/z* 40–500. The identified compounds were determined by comparing their mass spectra with those available in the NIST/EPA/NIH Mass Spectral Library and by matching their retention indices with published data [16,17].

2- CS (2000-3500cps) very high molecular weight was purchased from Glentham LIFE SCIENCES Ltd and PVA (molecular weight 14000 g/mol) from THOMAS BAKER (India). Glacial acetic acid (99.7%) was purchased from LOBA CHEMIE PVT.LTD Meanwhile, local grape seeds were collected from local markets, washed, dried, and ground into powder. Subsequently, grape seed extract (GSE) was prepared and converted into nanoparticles using solvent evaporation and ultrasonication techniques.

Preparation of Electro spun

Nanofibers 10wt% aqueous solution of PVA was prepared and 2wt% CS solution was prepared by dissolving CS in acetic acid water (90% v/v). The PVA / CS solution was mixed with 7:3 volume ratios., followed by a 0.5% (w/v) concentration of dried grape seed extract was added to the polymeric matrix, with continuous stirring for 24 hours at 50 °C. to ensure homogeneous distribution of the prepared nanomaterials. Load the prepared solution into a 5 mL syringe to start the electrospinning process under controlled conditions: voltage 20 kV, flow rate 1 mL/h, and a distance from the needle to the collector of 10 cm. Collect the nanofibers on aluminum foil, then dry at room temperature and store in desiccators until later use.

Experimental Animal Model

Adult male local rabbits, weighing between 2.5 and 3.5 kg, were acclimated to standard laboratory conditions with unrestricted access to food and water. Approval for the experimental procedures was obtained from the Institutional

Animal Care and Use Committee at the College of Veterinary Medicine, University of Basrah. In the first group, the mid-ventral side of each rabbit was exposed to UVB radiation (312 nm, 1 MED/hour) for 30 consecutive days. The chemo-biological measurements and histological analyses evaluated the level of damage in skin tissue after 30 days of chronic ultraviolet exposure, as well as assessed the extent of repair and regeneration after treatment of the damaged skin areas in

Table 1. GC-MS of grape seeds chemical compounds.

No.	Compound Name	Molecular Formula	Retention Time (RT)	CAS Number	Identity (%)	Notes
1	Cyclobutane, 1,2:3, 4-bis (propaneboronate)	$C_{10}H_{18}B_2O_4$	5.06 min	1000159-70-4	51.29 %	Boron-containing compound
2	Azido-2,4,4,6,6-pentamethylheptane	$C_{12}H_{25}N_3$	5.11 min	1000293-29-0	—	Organic azide, likely synthetic by-product
3	Bicyclo[4.2.1]nona-2,4,7-triene, 9-(phenylseleno)-	$C_{15}H_{14}Se_4$	—	—	—	Organo selenium compound
4	Cyclohexylmethylsilane	$C_7H_{16}Si$	5.19 min	2096-99-3	58.57 %	Industrial organ silane
5	Oleic acid, butyl ester	$C_{22}H_{42}O_2$	18.84 min	142-77-8	59.24 %	Fatty acid ester, plant-derived or lipid component

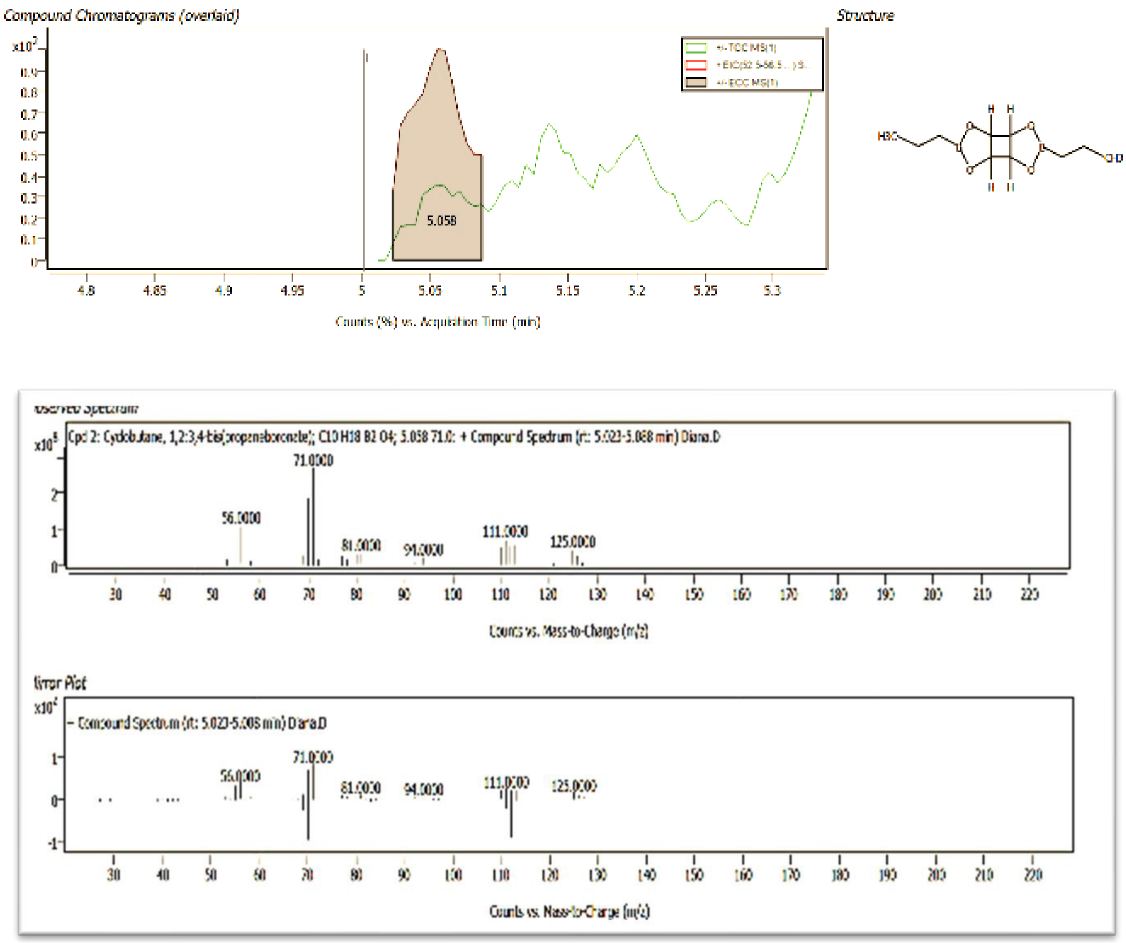

Fig. 1. Cyclobutane, 1, 2:3, 4-bis(propaneboronate).

the group treated with PVA/CS/GS nanofiber dressings for an additional 7 consecutive days following exposure. The damaged skin areas were dressed with the nanofibers daily, and then the level of regeneration and repair was evaluated for comparison with the negative control animal group that was not exposed to ultraviolet radiation during this period.

Histological Analysis

Skin tissue samples were collected from the experimental animal groups (treatment, processing, and negative control) and were immediately fixed in 10% neutral formalin solution. The samples were then processed through a graded series of ethyl alcohol and xylene, embedded in paraffin wax, and cut into 5-micrometer-thick sections using a rotary microtome. All our samples were stained using the conventional hematoxylin and eosin (H&E) stain. The histological condition was then assessed using a light microscope, and the tissue sections were photographed with a digital camera connected to the microscope at 10X and 40X magnification [18,19].

RESULTS AND DISCUSSION

A GC-MS/MS analysis

The sample indicated a complicated chemical composition. Five compounds out of 90 were chosen for comprehensive reporting due to their structural significance, library match quality (identity ≥50%), and possible biological or industrial relevance. The findings are summarized in Table 1. At RT 5.26 min, a new organ selenium molecule was identified (CAS 72065431, identity 65.14 %), possibly related to compound 3.

Key observations:

1. Retention periods varied from 5.06 min to 18.84 min, reflecting changes in polarity and molecule size.

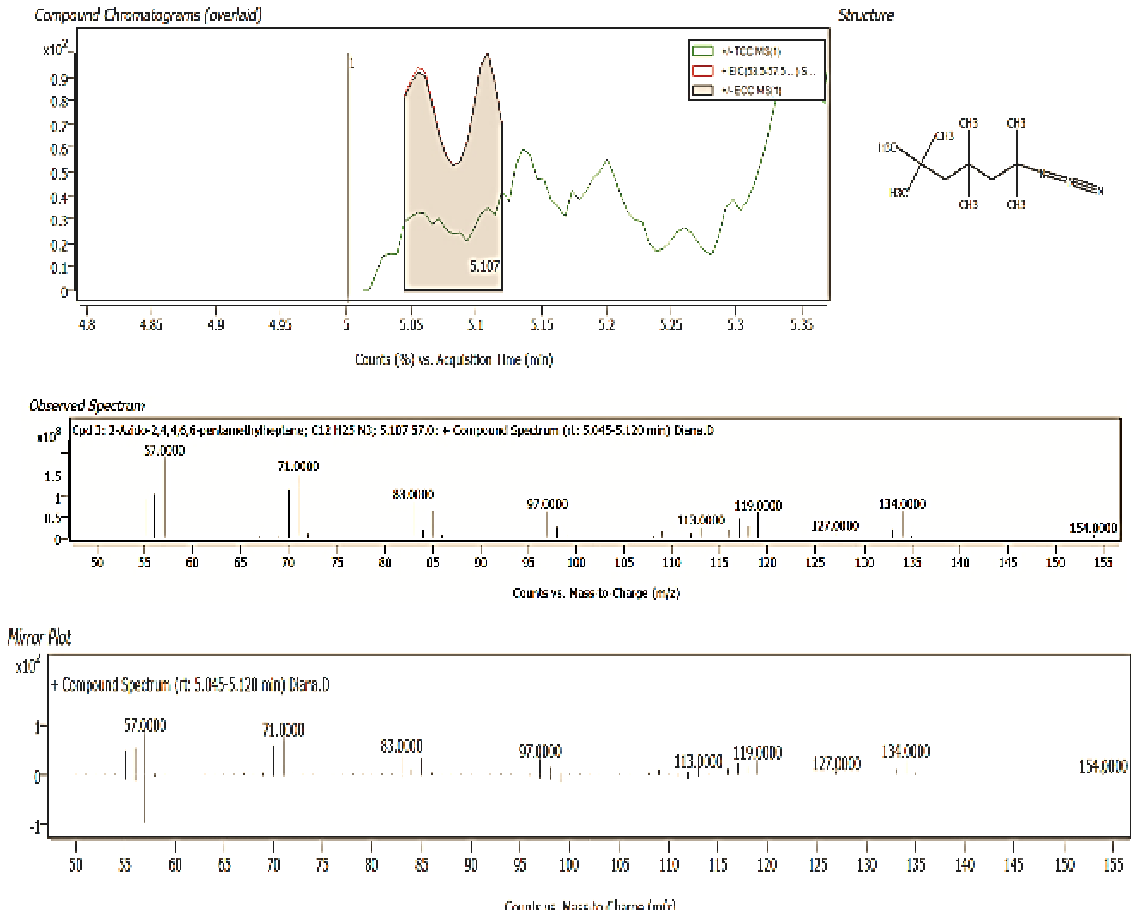

Fig. 2. Azido- 2, 4, 4, 6, 6-pentamethylheptane.

2. Identity percentages ranged from 51 to 65 percent, indicating questionable identification.

3. The sample contains a variety of chemical classes, including boron and selenium-containing compounds, an azide, a silane, and a fatty acid ester.

1. Chemical Compound Name: Cyclobutene, 1, 2:3, 4- bis (propaneboronate)

Molecular Formula: $C_{10}H_{18}B_2O_4$

Retention Time (RT): 5.06 min

CAS Number: 1000159-70-4

Identity (%): 51.29

Notes: A boron-containing chemical with potential antioxidant effects

2- Chemical Compound Name: Azido- 2, 4, 4, 6, 6-pentamethylheptane

Molecular Formula: $C_{12}H_{25}N_3$

Retention Time (RT): 5.11 min

CAS Number: 1000293-29-0

Notes: An organic azide molecule, potentially a by-product of organic synthesis.

3- Chemical Compound Name: Bicyclo [4.2.1] nona-2,4,7-triene, 9(phenylseleno)-

Molecular Formula: $C_{15}H_{14}Se$

Retention Time (RT): 5.26 min

CAS Number: 72065-43-1

Identity (%): 65.14

Notes: An organoselenic chemical. Selenium exhibits antioxidant action

4- Chemical Compound Name: Cyclohexylmethylsilane

Molecular Formula: $C_7H_{16}Si$

Retention Time (RT): 5.19 min

CAS Number: 2096-99-3

Identity (%): 58.57

Notes: An organosilane chemical that is widely employed in industrial applications.

5- Chemical Compound Name: Oleic acid, butyl ester

Molecular Formula: $C_{22}H_{42}O_2$

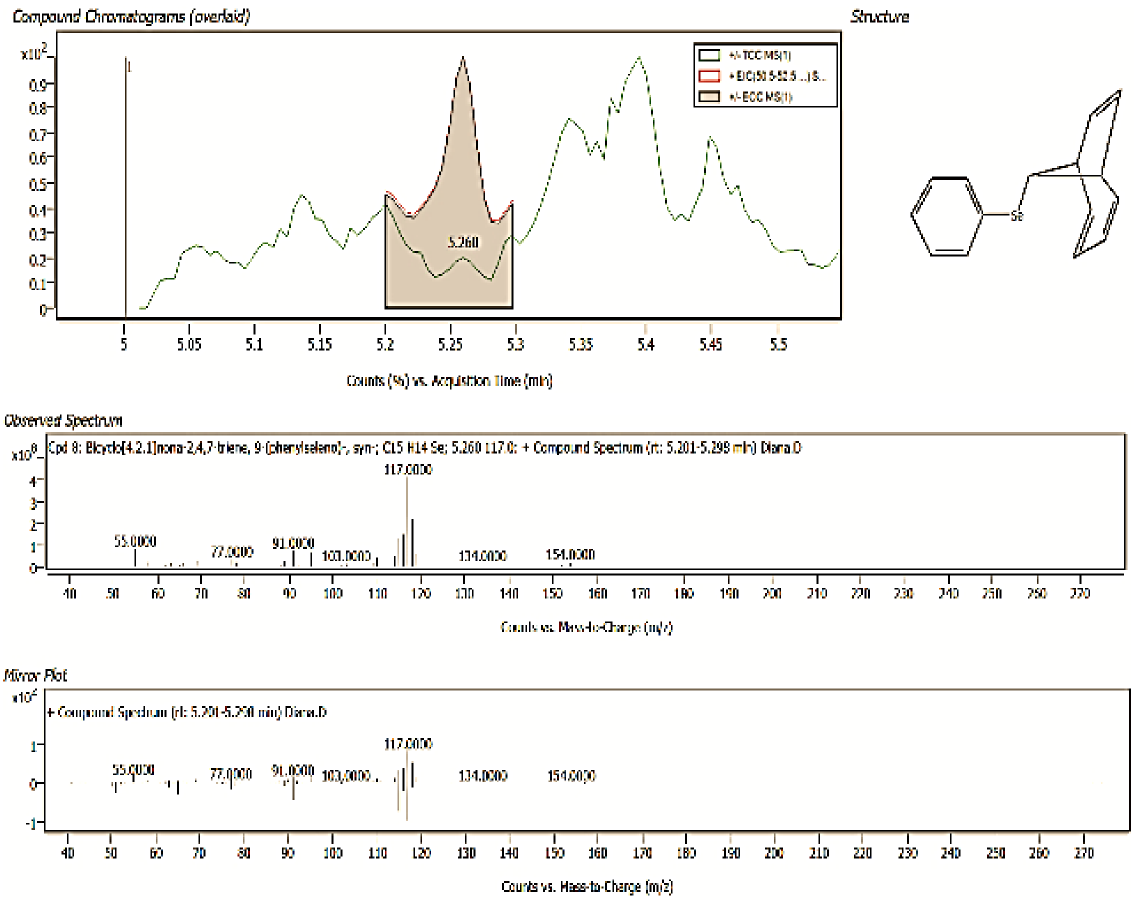

Fig. 3. Bicyclo [4.2.1] nona-2,4,7-triene, 9(phenylseleno).

Retention Time (RT): 18.84 min
CAS Number: 142-77-8
Identity (%): 59.24
Notes: Vegetable oils contain fatty acid esters, which are biologically important.

Five of the 90 compounds identified by GC-MS/MS analysis were selected for inclusion based on their structural significance, high match quality (library scores greater than 50%), and potential biological or industrial relevance. These chemicals belong to several chemical classes, including boron-containing molecules, azido derivatives, organo-selenium compounds, silicon-based structures, and fatty acid esters. Notably, the lack of oleic acid butyl ester suggests that the sample contains lipid- or plant-derived components. Similarly, the presence of organo-boron and organo-selenium compounds could indicate the inclusion of antioxidants or pharmacologically or biologically active components. The results shown lend weight to the concept that the specimen comprises a diverse range of chemicals with potential medicinal or biological functional applications.

Histological results

Control group (Fig.6) revealed a normal epidermis of stratified squamous keratinized epithelium and a dermis of thick irregular connective tissue with hair follicles, sebaceous glands, and blood vessels. The overall tissue architecture was well conserved, with no pathological changes, indicating the typical structural integrity of healthy skin.

The observation of rabbit skin subjected to UV. Radiation for 30 consecutive days revealed the epidermis exhibit a cute hyperkeratosis, parakeratosis necrotic keratinocyte acantholysis (Fig. 7). Other abnormalities of the epidermis included advanced solar elastosis, keratinocyte invasion into epidermis, epidermal atrophy.

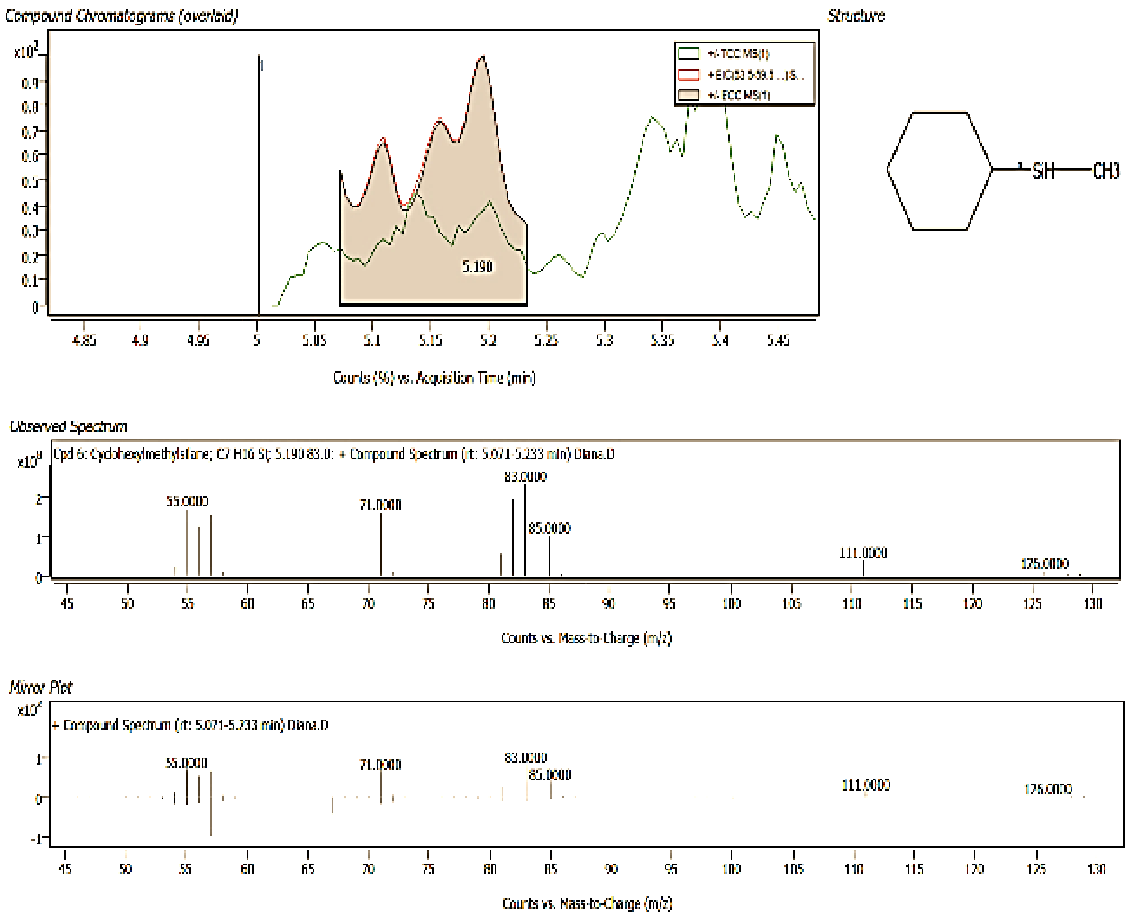

Fig. 4. Cyclohexylmethylsilane

Histological investigation of rabbit skin subjected to UV radiation and then treated for 7 days with chitosan nanofibers containing grape seed nanoparticles indicated nearly complete restoration of normal skin architecture. The epidermis was a typical stratified squamous keratinized epithelium, but the dermis was dense, irregular connective tissue rich with sebaceous glands, hair follicles, and blood vessels. Only mild dermal edema was observed in a few locations; however, the general tissue architecture mirrored that of healthy skin. These findings show that the treatment had a significant therapeutic effect, promoting nearly complete structural repair.

The chemical analysis of the grape seed extract by the GC–MS/MS method showed that it contained various chemically active compounds. One of them was found to be 1,2,3,4-cyclobutane, which was a major precursor of boron. The compounds of boron have received a lot of attention because of their biological and medical interest, especially their antioxidant property and their capacity to regulate enzymatic pathways. The presence of this compound as reactive oxygen species (ROS) scavenger implies that the extract has molecules that can affect the redox balance of the cell at a relative concentration of 51.29% [20].

These compounds can also be used in pharmaceutical preparations with the purpose of abundant, such as azido-2,4,4,6,6-pentamethylheptane. Organic azides are mainly not found in nature and are highly reactive

and may be labile, thus caution has to be observed when working with them during analyses and experiments. Further, it was found to have bicyclo[4.2.1]nona-2,4,7-triene, 9-(phenylseleno)-, an organ selenium compound. Selenium is an element that has well-documented

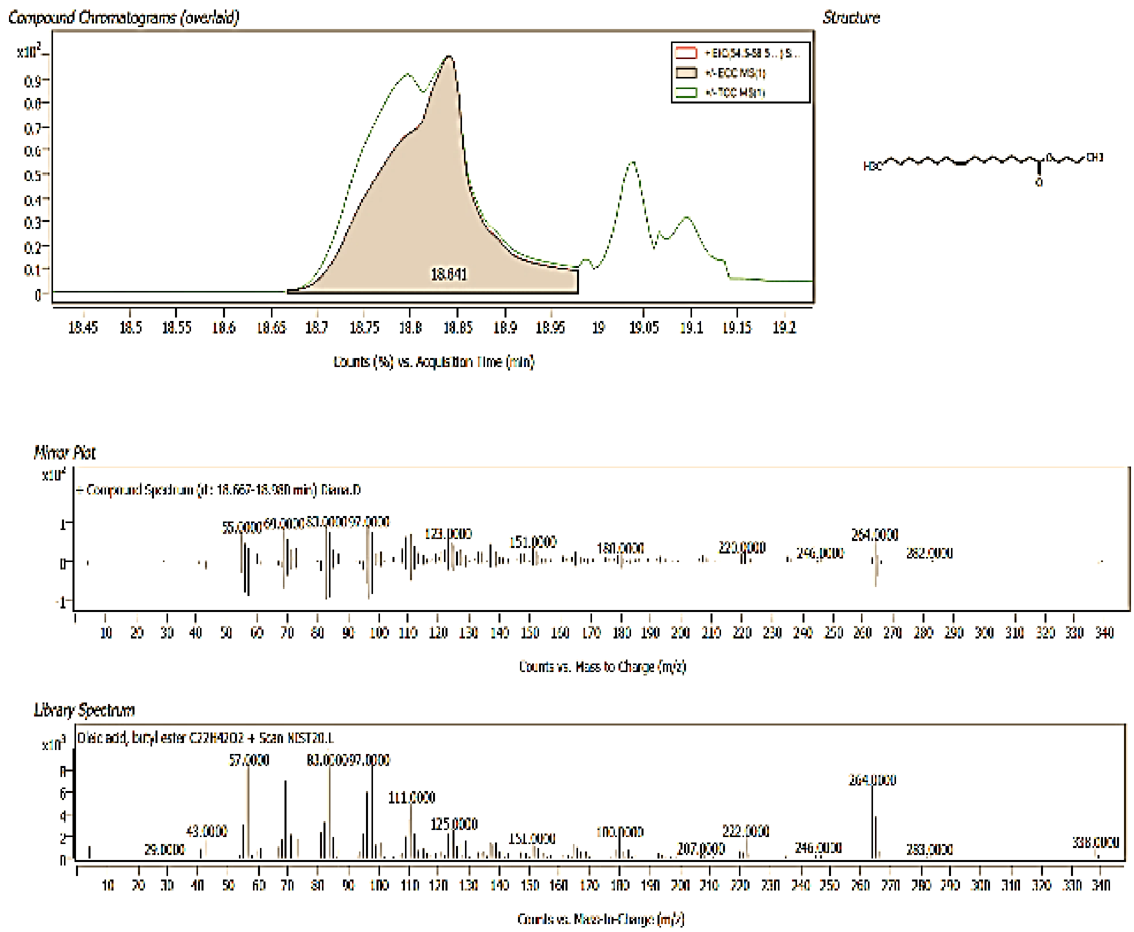

Fig. 5. Oleic acid, butyl ester.

antioxidant, chemo preventive, and therapeutic effects, which makes it a biologically important element. It is important in controlling the oxidative-reductive balance and safeguarding biomolecules against oxidation. It has been shown that the organoselenium compounds have high potential as agents of cancer prevention and reduction of oxidative stress [21,22].

The other compound was the cyclohexylmethylsilane, a binding agent and a biologically active intermediate. Such compounds can play a role in bioactivity, but their presence can have an impact on the extraction of other analytes, and the chemical environment can have an impact on the general analytical profile of the sample [23].

A fatty acid ester of Butyloleic acid, which is commonly present in plant-based and lipid-rich materials, was also identified. The fatty acid esters are vital to the biological systems as they aid in energy storage and in membrane structure. The hydrophobic nature and relatively high molecular weight of this compound contribute to its long shelf life of this compound. Grape seed extract would also have lipid components, which can mediate interactions with bioactive molecules [24]. The combination of these findings suggests that grape seeds are a complex assembly of chemicals that occur naturally. The presence of plant-based lipids with the bioactive components, such as boron- and selenium-containing compounds, demonstrates their potential to be utilized as pharmaceutical or food additives.

Besides, the presence of industrially relevant compounds, i.e., selenites and azides, highlights the chemical diversity of grape seeds. All in all, this discussion shows that the chemical content of grape seeds is complex and requires more research on biological and functional characteristics.

30-day chronic exposure to ultraviolet (UV)

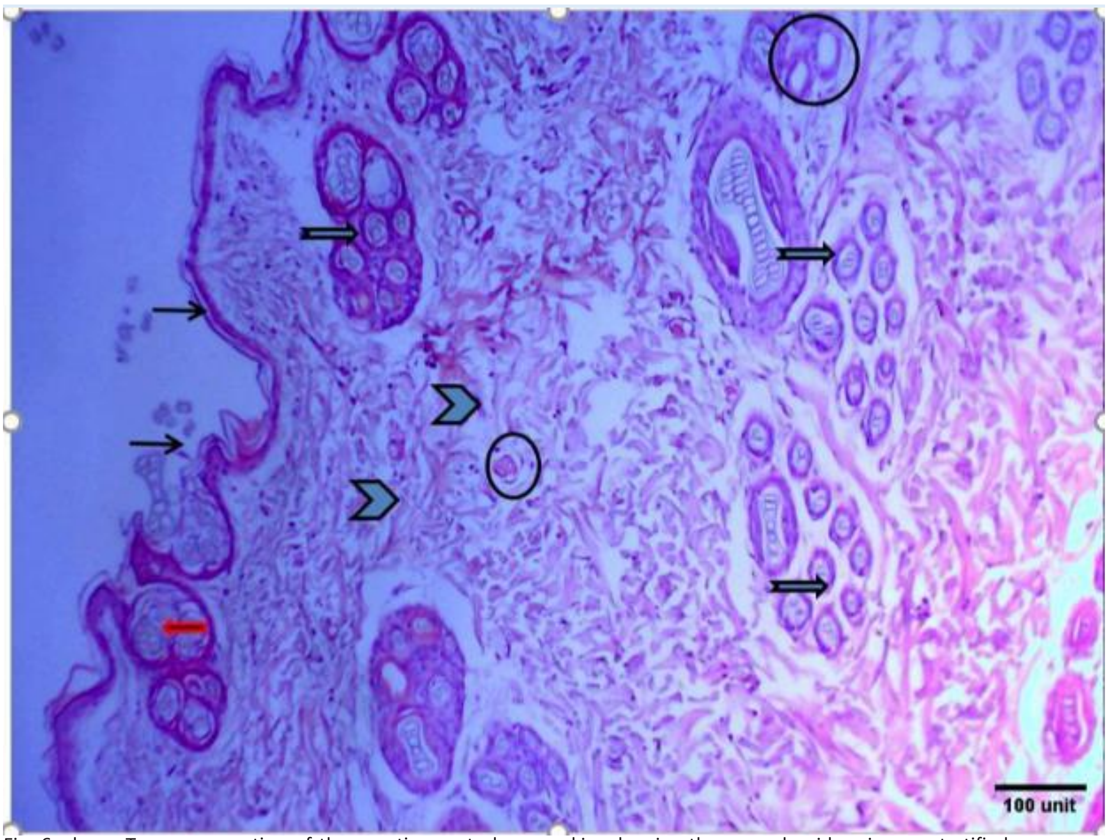

Fig. 6. shows Transverse section of the negative control group skin, showing the normal epidermis as a stratified squamous keratinized epithelium (black arrow). The dermis consists of dense, irregular connective tissue (arrowhead), which contains blood vessels (circle), hair follicles (thick arrow), and sebaceous glands (red thick arrow). This image illustrates the typical structural integrity of healthy skin (H and E stain - 100X).

light followed by a 7day post-exposure period, produced severe histopathological changes, which suggests that the natural skin architecture could not be repaired during the recovery period of 7 days. UV-exposed epidermis of both groups showed parakeratosis, vacuolar interface degeneration, subepidermal bullae formation, and keratinocyte loss, indicating direct cytotoxicity and apoptosis by UV, which was previously shown by Wang et al. [19]. It had a dermal alteration characterized by intense collagen degradation, necrosis, loss of architecture, edema, which are in line with extracellular matrix destruction mediated by the use of matrix metalloproteinase and vascular damage by UV, as postulated by [4] and [25]. Moreover, hypertrophy, hyperplasia, and focal necrosis were observed in adnexal structures, such as the outer root sheath of hair follicles and sebaceous glands, as well, which is evidence of maladaptive changes in tissue remodeling due to chronic exposure to phototoxic stress [26,27]. Persistence of inflammatory cell infiltrates is yet another indication of the existence of the persistent pro-inflammatory microenvironment, which exacerbates tissue degeneration and delays healing. Taken together, these results prove the cumulative and prolonged character of the UV-induced skin damage.

Use of grape seed nanoparticles in the application of chitosan nanofibers led to a significant increase in the wound healing of skin that had been damaged by ultraviolet (UV) radiation. This finding shows that the therapeutic effect of chitosan nanofibers is increased by the addition of grape seed nanoparticles in comparison with the use of the latter on its own. In those animals where the radiation of UV was applied during 30 days of the row and then7 days of treatment, the epidermis to a great extent returned to its usual stratified keratinized structure. Besides that, the dermis also exhibited great collagen repair and slight residual degradation. This biological activity of grape seed polyphenols can be the reason behind such a recovery, as these molecules have been proven to neutralize reactive oxygen species, inhibit pro-inflammatory cytokines, and prevent cell death in keratinocytes after UV irradiation, which justifies prior studies on

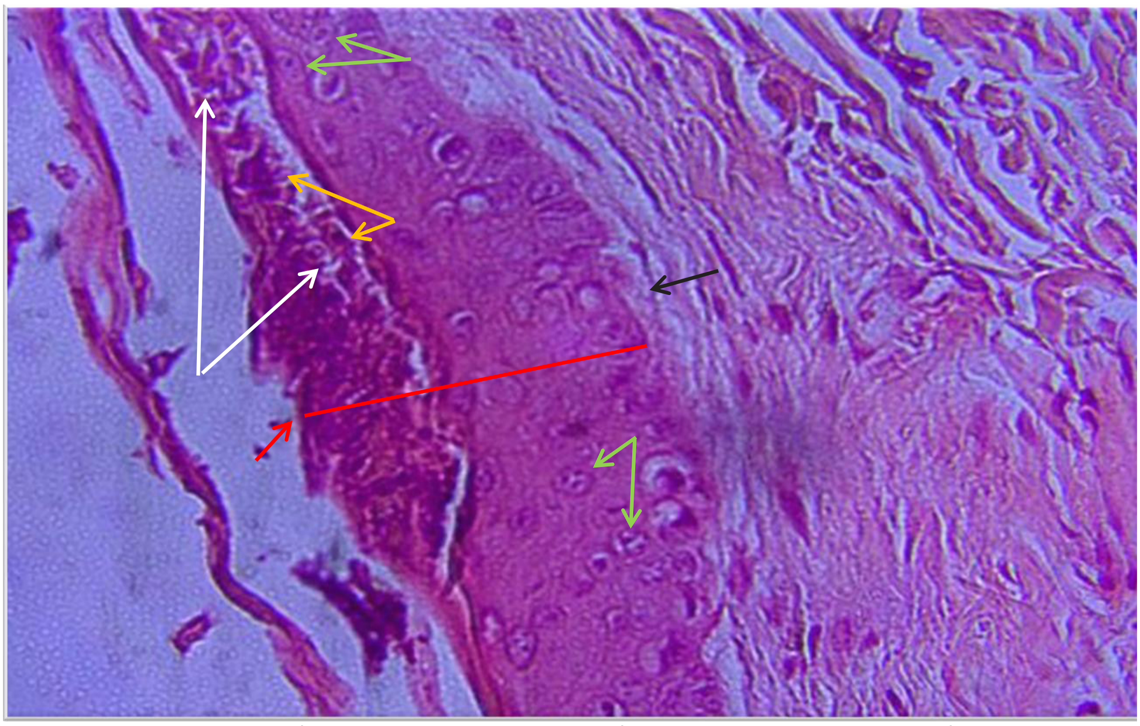

Fig. 7. Shows transversal section of rabbit skin exposed to UV radiation for 30 days. The tissue exhibits signs of acute hyperkeratosis (red arrow), parakeratosis (yellow arrow), hypogranulosis (black arrow), apoptosis keratinocytes (green arrows), acantholysis (white arrows), necrotic keratinocytes (orange arrow). This sample was stained using E and H and observed at 400X magnification.

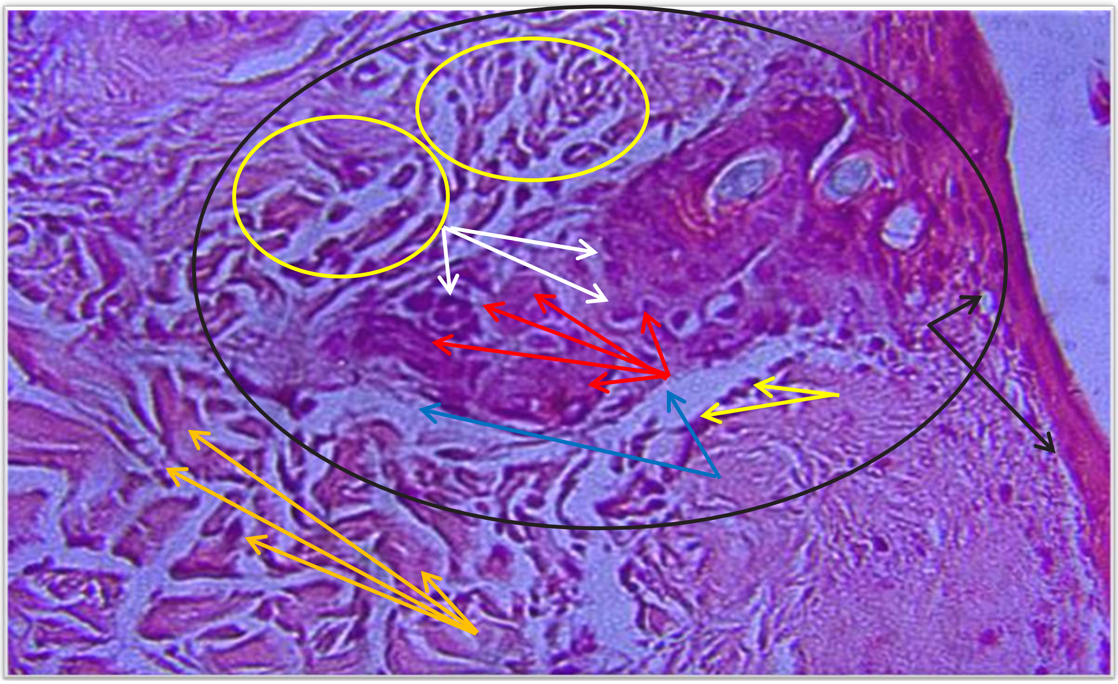

Fig. 8. Shows transverse section of rabbit skin exposed to UV radiation for 30 days. The tissue exhibits signs of parakeratosis (blue arrow), Epidermal Atrophy (green arrow), keratinocyte invasion into the dermis) black circle), dysplastic transformation of keratinocytes (red arrows), nuclear atypia (white arrow), chronic Inflammatory Infiltrate (thick yellow arrows), dermal edema (gray arrows), collagen degeneration and necrosis (orange circle), vacuolization at the basal layer (black arrow), and solar elastosis (yellow circle). This sample was stained using E and H and observed at 400X magnification.

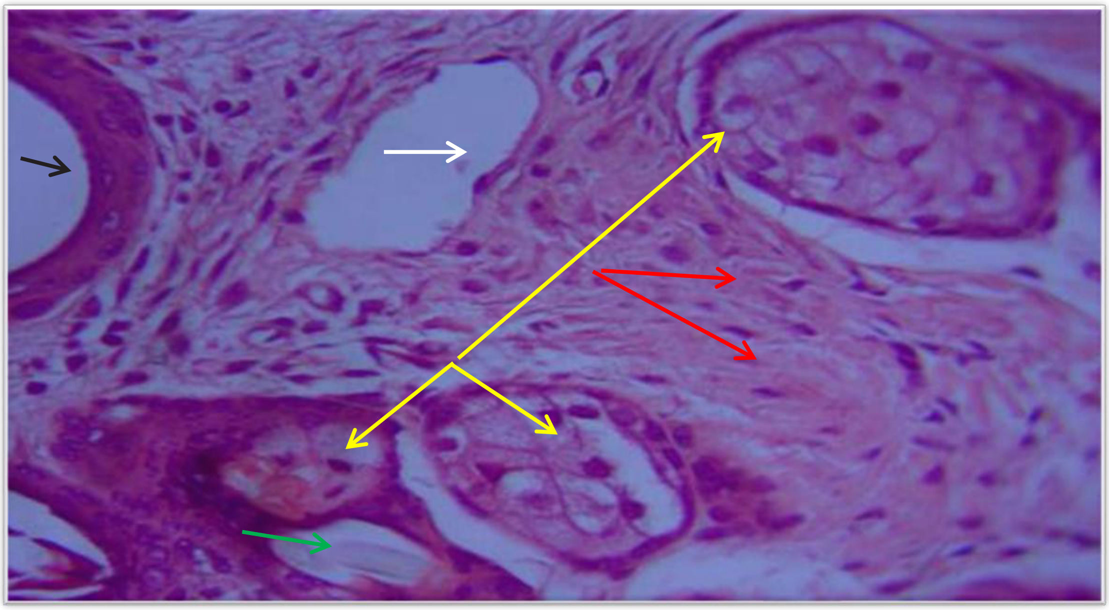

Fig. 9. Shows transverse section of rabbit skin exposed to UV radiation and treated for 7 days, showing the restoration of the normal histological architecture, which is represented by a normal epidermis as a stratified squamous keratinized epithelium (black arrow). The dermis is composed of dense, irregular connective tissue (red arrow), which contains sebaceous glands (yellow arrow), hair follicles (green arrow), and blood vessels (white arrow). Generally, the image depicts the typical structural integrity of healthy skin histology (H and E stain - 100X)

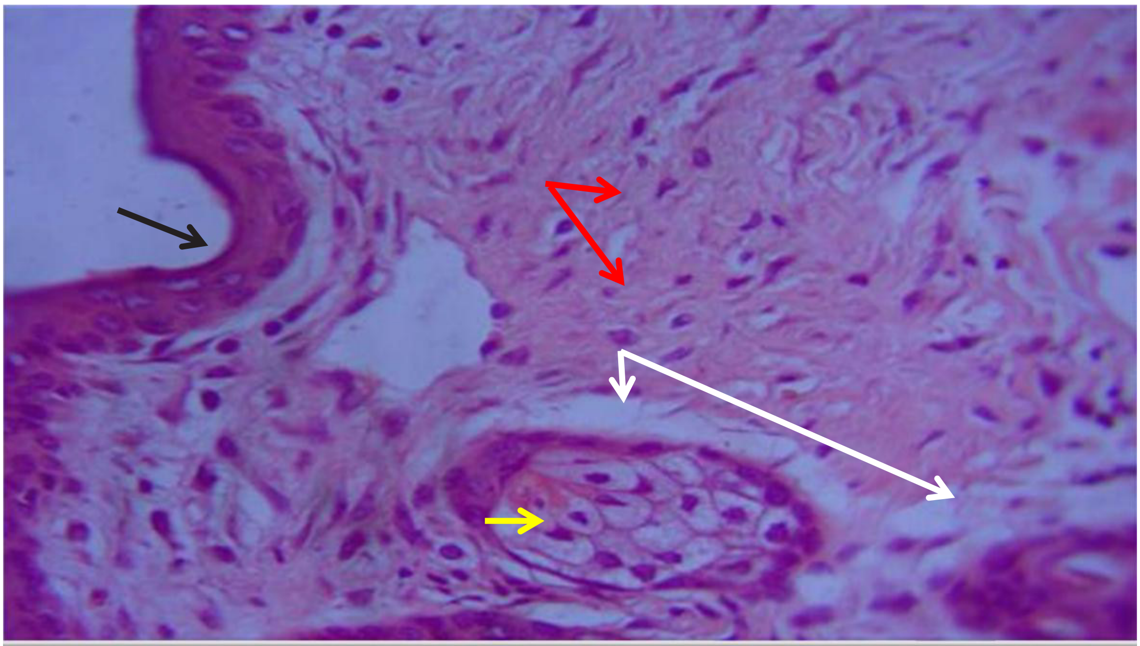

Fig. 10. shows transverse section of rabbit skin exposed to UV radiation and treated for 7 days, showing the restoration of the normal histological architecture, which is represented by a normal epidermis as a stratified squamous keratinized epithelium (black arrow). The dermis is composed of dense, irregular connective tissue (red arrow), which contains sebaceous glands (yellow arrow) and blood vessels (blue arrow). Only slight dermal edema is observed (white arrows). Generally, the image depicts the typical structural integrity of healthy skin histology (H and E stain - 100X).

photoprotection. It is also suggested that chitosan may be of significant use in promoting epithelial repair and facilitating migration of immune cells that are involved in promoting the stabilization and reorganization of the extracellular matrix. In the condition of 30 days UV radiation and 7 days consecutive treatment, skin recovery was almost complete and the dermal architecture and skin appendages almost resembled the morphology of healthy tissue. The impressive enhancement of collagen organization is an indication of efficient inhibition of collagen degradation by the action of the matrix metalloproteinases which are major factors contributing to the aging of the skin under the UV light.

Altogether, the results of the current research indicate that the chitosan nanofibers and grape seed nanoparticles have a synergistic interaction. Grape seed nanoparticles contribute to the strong antioxidant and anti-inflammatory activities, whereas chitosan offers a good environment and support tissue regeneration. The combination of this action propagates the repair process and indicates the significance of the duration of treatment as well as biomaterial design in reversing the skin damage caused by UV.

CONCLUSION

This study showed that chronic UVB exposure caused severe histopathological damage in rabbit skin, including hyperkeratosis, parakeratosis, epidermal atrophy, necrotic keratinocyte loss, dermal edema, collagen degradation, and distortion of skin appendages. These changes confirm the destructive effect of prolonged ultraviolet radiation on normal skin structure. Treatment with electrospun PVA/CS nanofibers loaded with grape seed nanoparticles markedly improved the injured skin. After 7 days of treatment, the epidermis and dermis showed near-normal architecture, with improved collagen organization and only minor residual edema. This suggests that the nanofiber scaffold supported tissue repair, while grape seed bioactive compounds likely contributed antioxidant and anti-inflammatory protection against UV-induced damage. In conclusion, the combination of chitosan nanofibers and grape seed nanoparticles demonstrated a promising synergistic effect in accelerating the healing of UV-damaged skin. The developed nanofibrous dressing may represent an effective biomaterial for skin regeneration and photodamage repair.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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