PREPARATION AND CHARACTERIZATION OF GLUTATHIONE LOADED POLYVINYL ALCOHOL ELECTROSPUN NANOFIBERS

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ABSTRACT

Reduced glutathione, or L-glutathione, is a tripeptide protein that occurs naturally in almost all cells of the human body. It is an antioxidant and plays a significant role in neutralizing oxidative stress. Oxidative stress is responsible for promoting many diseases and abnormalities in the body. This antioxidant decreases with time, and resulting in a number of disorders. To overcome these issues, experts recommend taking glutathione supplements. This study was aimed to design glutathione-loaded polyvinyl alcohol nanofibers, considering the properties of nanofibers that could be used as glutathione supplements to improve the deficiency of glutathione in the human body. With the electrospinning technique, poly (vinyl alcohol) loaded glutathione nanofibers were designed. The prepared nanofibers were characterized using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), and Ultraviolet-Visible Spectroscopy (UV–Vis) techniques. The antioxidant activity of the nanofibers was also determined; the activity indicated the eradication of free radicals.

Key Words: Glutathione loaded PVA, PVA electrospun nanofibers, Preparation and characterisation of Glutathione

INTRODUCTION

Human body is a combination of dynamic and complex systems, where every single cell is responsible for performing its work and playing its part in the system. All the systems result from the combination of these very tiny cells. Most of the normal and healthy cells in the body divide throughout our lives and replace themselves in a precise manner (1).

Humans are oxygen-consuming, so they are sometimes at risk of oxidative stress. Free radicals are exceedingly receptive synthetic compounds that can damage these cells. They are made with a loss or gain of an electron in a molecule. Free radicals are generally framed in the body and assume a critical role in many normal cellular processes; however, an increase in free radicals may harm every fundamental part of the cell. The harm to cells caused by free radicals, particularly the harm to DNA, may result in development and advancing diseases and other health conditions (1)

Free radicals are created as a by-product of metabolism. These free radicals wildly hunt for further molecules to stabilize in the body to latch onto; as a result, a single free radical produces another free radical. Consequently, a cascade of free radicals results in tissues injury(1). The body has several mechanisms to minimize radical-induced damage and repair damage, such as the enzymes superoxide dismutase, catalase, and glutathione peroxidase and glutathione reductase. Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. They inhibit oxidation, therefore preventing the production of free radicals (2, 3). Reduced Glutathione (GSH) is a naturally occurring abundant tripeptide protein. This protein is a highly reactive compound. Glutathione plays dynamic roles within a cell. Glutathione follows a simple mechanism, i.e. it donates electrons to free radicals, or molecules with unpaired electrons, to neutralize them and preventing them from causing cellular damage (4, 5).

Reduced Glutathione

Reduced Glutathione or L-glutathione, a naturally occurring abundant tripeptide protein made up of 3 amino acids: Glutamic acid, Cysteine and Glycine, is found in almost all cells. This protein is a highly reactive compound. It mostly found conjugated to other molecules and compounds through its sulfhydryl moiety. Glutathione plays dynamic roles within a cell. Glutathione is a cellular detoxifier that maintains the redox state, antioxidation, immune response modulation, xenobiotic and drug detoxification, and protecting cells from damage by environmental toxins, free radicals, carcinogens, bad fats and peroxides are some essential roles played by glutathione (4, 5, 6).

Reduced glutathione donates electrons to free radicals, or molecules with unpaired electrons, to neutralize them and to prevent them from causing cellular damage. It is the essential detox and safeguarding operator responsible for restoring antioxidants (7, 8). Glutathione, *"The Detoxifier"*, helps break down the adverse effects of environmental toxins. The liver has a high concentration of glutathione. The liver executes hundreds of functions; one such function is detoxification (4, 5, 7). Throughout the body, various immune functions are supported by glutathione, *"the Booster"*. T-cells cannot function properly without glutathione, also vitamins C and E need glutathione to function appropriately (8, 9).

Oxidative stress and Free radicals

Available literature reported that glutathione deficiency adds to oxidative stress, which, impacts the advancement of a number of disorders, including Alzheimer's disease, Parkinson's disease, liver dysfunction, cystic fibrosis, sickle cell anaemia, malignant growth, coronary artery disease, stroke, and diabetes (10). Oxidative stress in the kidneys may cause kidney issues or even kidney failure, contingent upon its seriousness (11).

Different studies have reported that the body makes less glutathione as it ages. In women, glutathione concentration decreases at the start of menopause and continues to remain lower. Delayed oxidative stress from low glutathione levels in older individuals can make the bones weaker and may add to osteoporosis. Cells drained of glutathione are vulnerable to harm. Low levels trigger a course that at last prompts cell damage and death, which quickens the process of ageing (12, 13). Decreased action of glutathione peroxidase and low degrees of glutathione are associated with high oxidative stress and an increased probability of heart attack. Cardiovascular illness is, to a greater extent, initiated by oxidative stress in heart tissues (14-16).

Glutathione keeps the liver sound by neutralizing the oxidative stress that can prompt liver illness. It is significant in detoxing the liver and ensuring its sulphur-rich antioxidant pathways. When tackled with damaging ingredients, the liver will produce more glutathione to overcome the harm (17-19). Inadequate antioxidant agents, including glutathione, might exacerbate cell death in the liver. As a result, it can cause fatty liver disease in both the individuals who alcoholics and the non-alcoholic individuals. Glutathione has improved proteins, enzymes, and bilirubin levels in the blood of people with alcoholic and non-alcoholic fatty liver disease (20). Several studies have recommended critical oxygen-free radicals (OFR) function in developing tumours and cancer (21).

Glutathione plays a fundamental role in almost every system of the human body. However, with the advancing age glutathione efficiency declines in human body due to many factors, such as eating unhealthy food and being exposed to a toxic environment for an extended period of time (1). To combat these issues, this study attempted to devise a way to design glutathione-carrying nanofibers. The distinctive properties of fibers have gotten extraordinary consideration from mainstream researchers as a reasonable contender for drug delivery applications. Nanofiber membranes from biopolymers can be used as a drug carrier. As a result, nanofibers provide significant bioavailability improvement (22,23).

The unique properties of fibers have received significant attention from the scientific community as suitable candidate for drug delivery applications. Their properties include high surface-to-volume ratio, high porosity, adjustable pore size and morphological similarity to the extracellular matrix (8-11). This study aimed to come up with a way to design glutathione-carrying nanofibers. Electrospinning is a technique used to design nanofibers. Electrospinning is a process in which a charged polymer jet is collected on a grounded collector.

MATERIALS AND METHODS

Materials

Reduced Glutathione: The drug L-Glutathione was purchased from bioWORLD Company, Dublin, USA.

Poly (vinyl alcohol): Poly (vinyl alcohol) (PVA) is a non-toxic, biodegradable, water-soluble synthetic polymer. It is used as an inactive ingredient that works as the medium for a drug or other active substance and as a surfactant for forming polymer-encapsulated nanofibers.

It is also considered a Generally Regarded As Safe (GRAS). Poly (vinyl Alcohol) was able to encapsulate glutathione. These were the reasons to use PVA as a polymer here (24), PVA was acquired from the lab.

Vitamin C: Vitamin C served the purpose of hindering the oxidation of glutathione due to environmental oxygen (25). CECON tablets (Vitamin C) were procured from Abott-Pharma (USA). Water served the role of solvent.

Methods

A method that has grown immensely popular for nanofiber production is electrospinning. Electrospinning is a process in which a charged polymer jet is collected on a grounded collector. Electrospinning involves using voltage to draw a jet of polymer solution from a syringe/ pipette source towards a collector. High voltage comes to aid in creating a repulsive force and charging the particles. Jet erupts from the tip of the syringe/ pipette as soon as the repulsive force overcomes the surface tension of the solution. High voltage also helps draw a fiber from the tip of the syringe/ pipette. As the charged jet accelerates towards regions of lower potential, i.e., the collector, the solvent evaporates while the entanglements of the polymer chains prevent the jet from breaking up. Collectors could either be rotating or stationary. Rapidly rotating collectors result in aligned nanofibers, while stationary collectors produce randomly oriented fiber mats. This results in fiber formation (26,27).

In its essential practice, this process consists of a pipette to hold the polymer solution, a collector and a voltage supply in the kV range. The jet is electrically charged, and the charge causes the fibers to bend so that every time the polymer fiber is twisted, its diameter is reduced. The fiber is collected as a web of fibers on the surface of a grounded target. Multiple trials were taken to prepare nanofibers. Both rotating and stationary collectors were used separately.

A 3ml solution was prepared, and substances were added through calculations. The tube solution added 0.27g of PVA (9% of total weight) to the 2.7ml water. The solution was stirred on the electronic stirrer at 100 rpms. 0.015grm of CECON, 10% of the drug's weight, was added to prevent the drug from oxidizing. No temperature was provided once the PVA was dissolved, and CECON was kept on the stirrer to dissolve completely. For almost 20 minutes, the solution was further kept for stirring. When the solution was back at room temperature, the 0.15grm, 50% of PVA weight, of the glutathione was added and kept further for stirring.

Once the solution was prepared, the polymer solution was filled in the syringe, the plastic tip was connected to the syringe, the anode, a copper electrode, was inserted in the polymer solution-filled syringe, and the positive terminal of the power supply was connected to the electrode. The collector end was set to collect the fibers; butter paper and aluminum foil were used for collecting purposes,

and the negative terminal from the power supply was connected to the collector plate. A black screen and light source were set to help focus or visualize the fibers. The syringe was firmly set on the handle stand. Once the setup was ready, the power supply was turned on at 12K volt.

The fabrication and synthesis of GSH-loaded PVA nanofibers were conducted at the Nanomaterials Research Group, Department of Textile Engineering, Mehran University of Engineering and Technology, Jamshoro, Pakistan.

Methods of characterization

The prepared fiber sheets underwent some methods and techniques for characterization and analysis. These methods of characterization were:

Scanning Electron Microscopy (SEM) provides information regarding the sample's composition and surface topography. The morphology of nanofibers was studied using a field emission scanning electron microscope (S-4800; Hitachi Ltd. Japan). SEM samples were coated with gold and examined at an accelerating voltage of 15 kV. The nanofiber diameter was calculated using ***ImageJ software***.

Fourier Transform Infrared Spectroscopy, or FTIR Analysis, is an analytical and diagnostic method used to recognize organic, polymeric, and, in some cases, inorganic materials. In this method, infrared light is used to perform scanning to perceive chemical properties. The chemical structure of nanofibers was analyzed using *FTIR spectroscopy* (Thermo Nicolet 5700, Thermo Fisher Scientific Inc. USA). For data analysis and curve fitting, Origin Pro 8 software was used.

X-ray diffraction is an X-ray-based technique where the material interacts with an x-ray beam of a specific wavelength. The beam gets dissipated, relying upon the crystal structure of the sample, and creates a plot with intensity elements as a function of 2 theta. The crystallinity of nanofibers was analysed using XRD, model D/max-IIB, and Rigaku RINT-2000 diffractometer with a source of filtered CuKα radiation. The measurement was performed at 40kV and 40mA with a diffraction angle of (2θ) between 5° and 70° at a scan rate of 4°/min. Origin Pro 8 software was used for data analysis and curve fitting.

Ultraviolet-Visible (UV–Vis) Spectroscopy was used to analyze the release behavior of GSH from GSH-loaded PVA nanofibers in PBS solution using a UV-1800-VIS Spectrophotometer (Shimadzu Corporation, Japan).

Antioxidant Activity was identified using the 2, 2-diphenyl-2-picrylhydrazyl hydrate(DPPH) assay. The DPPH stock solution in methanol was prepared. 1 mL of this stock solution was added to 3 mL of GSH solution. After giving the combination a thorough shake, the mixture was let to stand at room temperature for 30 minutes. Afterwards, a UV-visible spectrophotometer was used to detect the absorbance at 517 nm (28). Antioxidant activity was estimated by calculating the percentage inhibition ratio by the following formula;

$$\textit{Inhibition ratio (\%)} = (AC-AS)/AC \times 100$$

where AC was the absorbance of the control, and AS is the absorbance of the testing sample (GSH).

RESULTS

SEM Analysis: As stated earlier, SEM gives information related to surface topography. Figure 1 shows the SEM images of the designed GSH-loaded PVA nanofibers. With the aid of ImageJ software, the diameter of GSH-loaded PVA nanofibers was attained, and their average was found to be 57.99967 nm. In contrast, following the same method for neat PVA fiber sheets, the average diameter was calculated to be 25.5153 nm.

FTIR Analysis: FTIR gives information about chemical structure and behavior of a compound. The IR table is followed to understand the chemical bonding of the functional groups in the compound. This table tells the range of the energy at which different functional groups lie in IR spectra; this allows us to identify which group would change, i.e. absorbance of IR radiation at which wave—

ultimately allowing us to understand the presence of functional groups and their behavior in the compound. The data obtained from the FTIR spectroscopy is plotted both on OriginPro.
As shown in Figure 2, the deep and broad trough between 3500 and 3000 cm-1 represents the presence of alcohol, OH group, a fundamental element of PVA. The narrow and sharp groove just on the right of 3000 cm-1 shows the presence of the CH bond; the CH bond is common in both GSH and PVA. Another deep, narrow trough on the left of 1500 cm-1 reflects the C=O, amide presence, a bond in GSH. This result was analyzed by using IR tables.

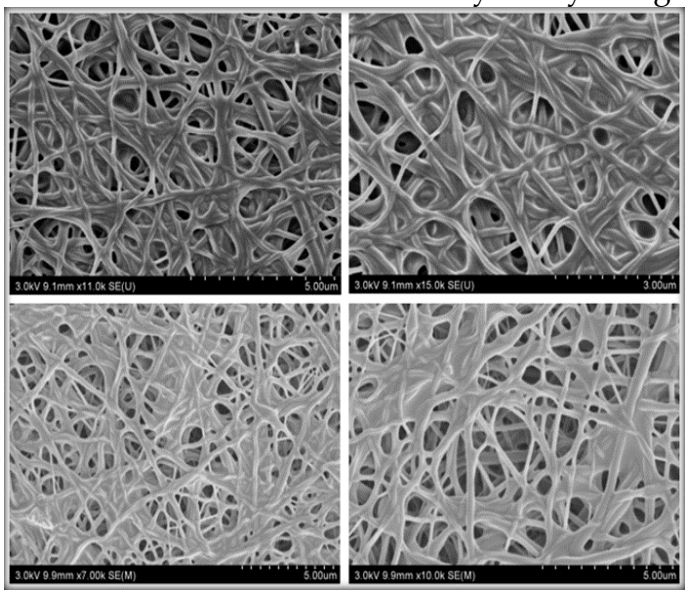

Figure 1. SEM Images of GSH-loaded PVA nanofiber

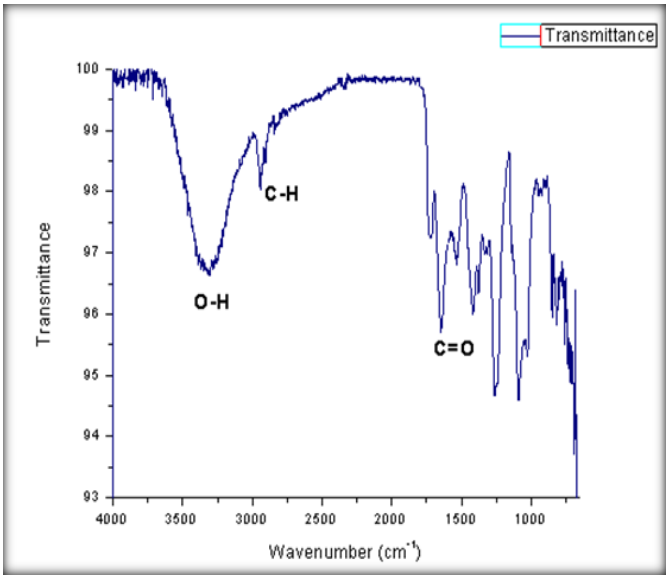

Figure 2. Data obtained from FTIR analysis plotted on OriginPro

XRD Analysis: XRD gives an understanding of whether the compound is crystalline or amorphous at the level of the unit cell. Figure 3 shows the XRD result and pattern of GSH-loaded PVA fibers, intensity vs. angle. The data obtained from the crystallography is plotted both on OriginPro. As shown in Figure 4, at X=10.08° there is a rise in the pattern of GSH-loaded PVA nanofibers; when 2θ varies from 18° to 26°, there is a rise in the XRD pattern. The pattern shows the dominance of the PVA, but at X=22.56°, the intensity is 1239, which is 93% (1324 is the 100% intensity).

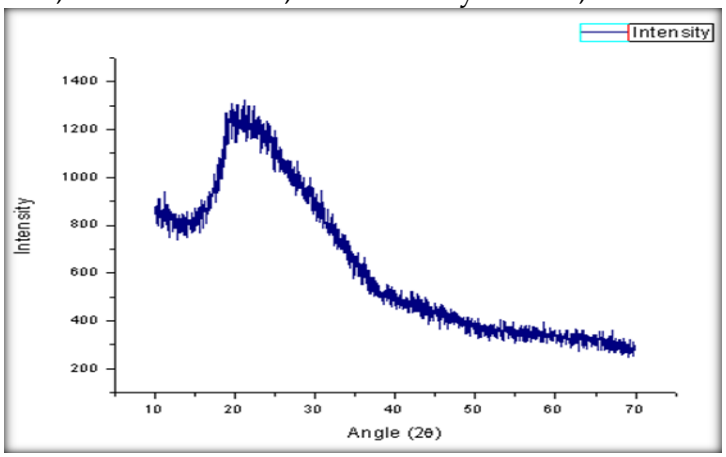

Figure 3. Data received from XRD analysis plotted on OriginPro.

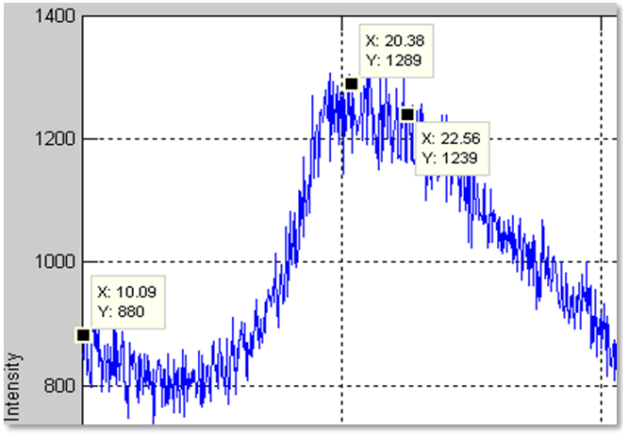

Figure 4. XRD pattern of GSH-loaded PVA nanofiber

UV-Vis Analysis: Figure 5 shows the UV/Vis result and pattern of GSH-loaded PVA fibers, absorption vs. wavelength. Phosphate Buffered Saline (PBS), a universally accepted salt solution used in biological research, was prepared by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g ofNa2HPO4, 0.24 g of KH2PO4 in 1-liter distilled H2O (Average pH 7.2 ~7.4). The GSH exhibited a maximum at 206 nm. For this purpose, a 10mg sheet of GSH-loaded PVA fibers is added to the 30ml Buffer solution.

The absorbance values were obtained from the prepared solution with the help of a UV-Vis Spectrophotometer. The absorbance value(s) of the solution is observed for 10 minutes, with an interval of 5 minutes. The data obtained from the spectroscopy is plotted on OriginPro. Antioxidant activity is presented in Figure 6 and Table 1.

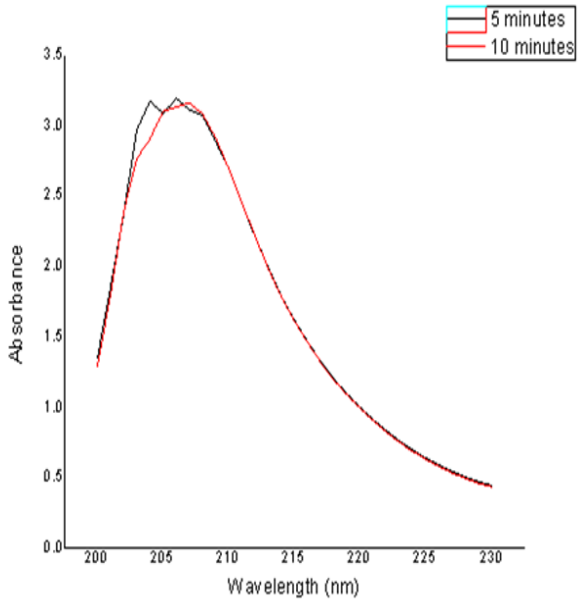

Figure 5. UV/Vis result and pattern of GSH-loaded PVA fibers

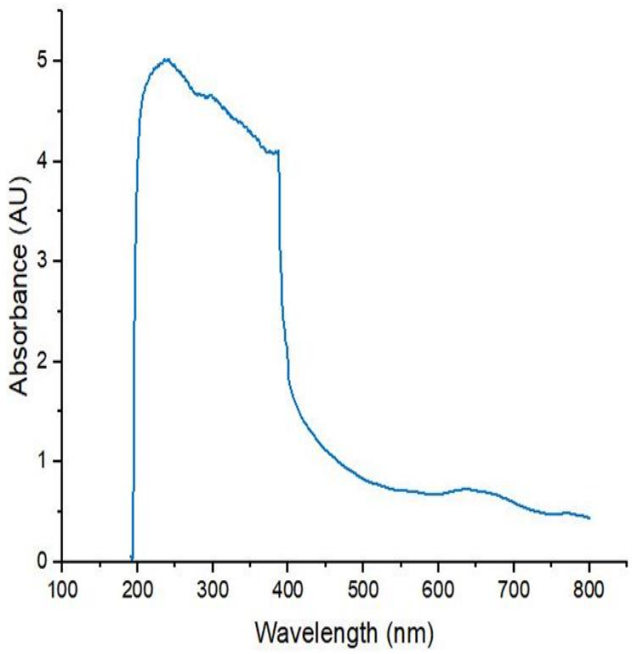

Figure 6. Antioxidant activity of GSH

Table 1. Radical inhibition ability of GSH

Sample type	Absorbance at 517 nm	% of radical inhibition
Control	**1.092**	**-**
GSH	**0.7807**	**28.5%**

DISCUSSION

Polyvinyl alcohol is a non-toxic and biocompatible polymer that degrades in the body; therefore, it is regarded as a safe material for research purposes. Due to its properties to form chains, it has been generally used as a nano-carrier for many applications. Because of these abilities, polyvinyl alcohol is used as a carrier for glutathione (29,30)

Glutathione is the most dominant antioxidant. It is continually at work in every single cell of the body. Nevertheless, the degree of glutathione in the body diminishes as part of the natural ageing process. Levels are affected adversely when the body is under stress from ailment; prolonged exposure to toxic substances additionally decreases glutathione advantages to the body. Boosting the body's capacity to yield glutathione with nanofibers will give numerous medical advantages, including decreased oxidative stress (31, 32).

Different drugs of abuse (for example, cocaine and alcohol) increment the generation of responsive oxygen species. These, thus, can modify the brain and cause harm. A few studies propose that expanding glutathione can help defeat addictive practices, which span from eating disorders to alcohol/drug abuse. Expanding antioxidant defense not only ensures the brain but also helps detox destructive substances from the body. Chronic alcohol abuse decreases glutathione in the liver. Rising

glutathione levels improved liver capacity during restraint. Liquor misuse likewise expands oxidative stress in the lungs, which can frequently promote diseases, for example, pneumonia. Glutathione might protect the lungs by lessening oxidative stress (33, 34)

In an investigation of 20 individuals with a chronic kidney infection on hemodialysis, glutathione improved kidney work (16). As suggested by Huang et al., renewing glutathione levels may slow the maturing procedure, toughen the bones, and counteract age-related decay (20). Studies have also shown that glutathione can lessen free radicals and, thus, may avert stroke or heart attack (23). Glutathione was most effective when given to individuals with fatty liver disease intravenously (30).

The lower degree of glutathione can build inflammation in the airways and cause asthma. In mice with asthma, expanding glutathione with NAC brought down inflammation and amended the manifestations. Chronic Obstructive Pulmonary Disease (COPD) is a lung illness brought about by long-term oxidative harm. Growing glutathione reduces free-radical lung harm, diminishing the probability of evolving COPD (35, 36)

In numerous chronic diseases, weak immunity and an amplified degree of infections are connected to low glutathione levels. The chronic inflammation brought about via autoimmune sicknesses can build oxidative stress. These maladies incorporate rheumatoid arthritis, celiac disease, and lupus. As per studies, glutathione lessens oxidative stress by decreasing the body's immunological reaction. Autoimmune diseases assault the mitochondria in cells. Glutathione attempts to ensure mitochondria’s safety by dispensing free radicals (37, 38).

Individuals with sleep apnea have significant levels of oxidative stress and, thus, exhausted glutathione levels. In one examination, increasing the levels improved their resting quality. The leading causes of complete loss of sight are glaucoma and cataracts. As oxidative stress is responsible for both, improving glutathione levels may help protect the eyes (39, 40).

Oxidative stress brings down glutathione in individuals with skin acne. Expanding glutathione levels may clean a person's acne by neutralizing oxidative stress and encouraging skin recovery. Glutathione lightens the skin in healthy women. It decreases the movement of skin cells that make dark pigments (melanin). Glutathione may help even out the presence of dark skin patches that show up with maturing. Various personal-care items containing glutathione are promoted for their alleged skin-brightening impacts. These items incorporate cleansers and creams. In any case, a few people take glutathione supplements for skin-brightening (15, 41-42).

In different studies, glutathione has been found to attack cancer cells and even reduce the contrary effects of other cancer treatments. A study showed the effects of glutathione in ovarian cancer cells. According to the published study, it was observed that IV glutathione triggered the death of cancer cells. The study results show that extracellular glutathione caused cancer cells' apoptosis (cell death) by triggering DNA damage in cancer cells (4). Studies are going on to highlight different roles played by glutathione that finally control tumour evolution and improve the use of glutathione-based drugs to specifically target this detoxifying system in cancer treatment to increase therapeutic response (43).

CONCLUSION

Glutathione plays a vital and dynamic role in almost all the functions of the human body. Nevertheless, with age and an unhealthy diet, the levels of glutathione decrease with time. In such cases, glutathione supplements are recommended. This study synthesised the PVA nanofibers loaded with GSH due to the myriad benefits of the nanofibers over conventional medicines. Further studies are required to explore clinical benefits of GSH nanofibers.

CONFLICT OF INTEREST:

Authors declare no conflict of interest

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The study did not receive any external funding
ETHICAL APPROVAL:
The study was approved by local Research Ethics Committee.
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