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Antioxidant Based Wound Healing Effects of Apple and Onion Peel Extracts Obtained from Sustainable Biological Resources

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Abstract: *Wound healing is a very complex phenomenon that includes cellular migration and maintaining a balance in the levels of oxidative stress. An elevated level of reactive oxygen species may induce poor healing rates during wound healing. Hence, there is an increasing importance in research regarding the potential use of natural and biological products in wound healing. A study is conducted to assess, within an in vitro setting, the wound healing and antioxidant properties of a combination extract derived from the peels of apple and red onion on fibroblast cells. In a mechanical wound model established within an L929 fibroblast cellular line, various doses (15, 60, 125, and 250µg/mL) of a combination extract impact on fibroblast cellular migration and rates of wound healing was analyzed for 24, 48, and 72 hours. The activity levels of superoxide dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) enzymes, and Malondialdehyde (MDA) content, have also been analyzed simultaneously. The findings indicate that the combination extract, used as 60µg/mL, enhanced fibroblast cellular migration, boosted antioxidant activity, and lowered MDA content, which, at larger doses, has revealed cytotoxic properties. These data indicate that this extract combination might be an onboard natural biotherapeutic that heals wounds at an appropriate dose.*

Keywords: *L929, Apple Peel, Onion Peel, Wound Healing, Antioxidant Activity.*

1. INTRODUCTION

Wounds are an important public health problem, with impairment of skin integrity having dire effects on the quality of life. In contrast, wound healing is a dynamic process of repair that includes different stages, such as hemostasis, inflammation, proliferation, and tissue maturation. Impaired cycles lead to the development of chronic wounds, which may further be infected by opportunistic pathogens, including *Staphylococcus aureus* and *Candida albicans*. Certain toxins secreted by microorganisms suppress the process of tissue repair and contribute to a delay in the healing process. So far, treatment modalities used have only limited efficacy and are ineffective against resistant microorganisms.

This has accelerated the search for alternative treatments; thus, interest in reliable, cost-effective, and natural therapeutic agents is growing, and plant-based compounds appear to be very attractive candidates for wound healing and infection control (Addis et al., 2020; Mounir et al., 2023).

Onion (*Allium cepa* L.) is an important plant species that serves as a valuable source of numerous bioactive compounds, including phenolics, flavonoids, thiosulfinates, organosulfur compounds, and anthocyanins. Antioxidant properties of onion rely, in major aspects, on flavonoids. Phytochemical studies have made it clear that onion contains a minimum of 25 different derivatives of the flavonols and that quercetin and quercetin derivatives account for 80-95% of the total content of flavonoids (Puišo et all., 2023). In a scientific study noted in the literature, red and yellow onion skin extracts were thoroughly assessed regarding their biological properties, and their in vivo efficacy in wound healing was investigated in excision and *C. albicans* induced skin infection models. As per the outcome of that scientific study, red onion skin extracts possess potent antibacterial properties, restrict the development of skin infections caused due to *C. albicans*, and also possess the ability of tissue healing components due to their rich content of quercetin and anthocyanin derivatives. In in vivo studies performed to investigate their

regenerative efficacy, it was made clear that yellow onion skin extracts possess the highest efficacy of tissue regeneration. The results derived from research conducted by researchers indicate that onion peel extracts exhibit potential wound-healing properties through which tissue regeneration is stimulated due to the inhibition of microbial infections and inflammation at the cellular level. Therefore, these onion peel extracts have potential therapeutic uses for wound treatment (Mounir et al., 2023).

The notable increase in fruit output in recent years has led to greater interest in the peel components of fruits, which are commonly disposed of as trash without any examination of their potential. For this study, apple skins and onion skins were chosen as research materials due to their high biological content. The apple is a common fruit cultivated on a global scale; it is an inexpensive ingredient of food, very hygienic, and has high biological content in form of phenolic compounds. The data from the Food and Agriculture Organization (FAO) indicates that production of apples worldwide per year is 75 million tons. Such high potential exists for apple skins to qualify as a biological resource.

Apples are one of the more biologically important fruits because of their high content of polyphenols, and their rich phytochemical components. The regular polyphenol groups found in apples are dihydrochalcones, hydroxycinnamic acids, anthocyanins, flavan-3-ols/procyanidins, and flavanols. The flavonoids, featuring derivatives of catechin, epicatechin, and procyanidin, possess greater antioxidant potential because of a higher redox potential. The phytochemicals found in apples are not distributed evenly throughout the fruit. The flesh of the fruit contains mostly catechins, phloretin glycosides, caffeic acid, procyanidins, and chlorogenic acid, where as the peel also contains flavonoids such as quercetin and cyanidin glycosides besides these compounds. And these bioactive compounds were mentioned to contribute to prevention from various chronic diseases like cardiovascular disease, colon cancer, diabetes, and obesity; they act against proliferation, inflammation, carcinogenesis, and oxidation (Shehzadi et al., 2020).

In literature, studies on the effects of apple skin extract on peripheral neuropathy in type 2 diabetic patients and delayed wound healing have been explored. The topical use of hydrocolloid films comprising a concentration of 5-20% of apple skin extract in diabetic rats resulted in a dose-dependent decrease in wound size and a greater degree of wound contraction. Wound contraction plays a pivotal role in the healing of damaged tissue. The results of the study revealed that flavonoid-rich extracts of apple skin facilitated wound healing by enhancing hexosamine and hydroxyproline expression and improving tensile strength of tissue in diabetic rats. The results provide evidence of the efficacy of apple skin extract as a novel drug target in counteracting delayed wound healing in diabetics (Lesperance et al., 2006; Kamdi et al., 2021).

Such production of synthetic dyes using petrochemical-based intermediates immensely affects the ecosystem. The wastewater generated during the various production processes, if discharged into natural water sources, releases toxic chemicals into the environment, increasing the potential for environmental pollution and global warming processes (Jahangiri et al., 2018). Hence, moving toward recycling, natural-based dyes is increasingly crucial not only for environmental sustainability but also for possible biological activities.

The principal reasons for the acceptance of dyes derived from natural sources presently are their non-toxicity to living organisms, high biocompatibility, and ability to respond to various biological properties. The biodegradability of dyes derived from natural sources such as plant, fungus, and lichen is high in comparison to that of dyes derived from synthetic sources and finds eco-freedom to a larger extent. Among the various natural dyes, plant dyes find the highest acceptance and are derived from plant sources such as roots, fruits, tree bark, leaves, and shells. A large number of the plants used as sources of dyes also come in the medicinal plant category, and a number of them, according to the literature, possess a marked anticrobial property. Various plant dyes, such as those of high naphthoquinone content like henna, walnut, and alkanet, have shown their marked antibacterial and antifungal properties. In a study performed by Singh and co-authors, the anticrobial properties of optimized natural dyes in powder form derived from natural sources like *Acacia catechu* (L.f.) Willd., *Kerria lacca*, *Rubia cordifolia* L., and *Rumex maritimus* were examined, and marked anticrobial properties were shown by the dyes (Chengaiah et al., 2010; Jahangiri et al., 2018;

Arroyo Figueroa et al., 2022). Literature also reports that botanical dyes could equally be sourced from the peels and seeds generated as waste products from agriculture; pomegranate peels, onion skins, leaves of eucalyptus, walnut shells, peanut shells, strawberries, indigo seeds, and sunflower seeds are some of the popular ones used for this purpose (Mohan et al., 2020).

Malondialdehyde (MDA), a cytotoxic lipid peroxide, is produced as a consequence of the oxidation of polyunsaturated fatty acids. MDA, generated by the oxidation of phospholipids in the cell membrane, can impair cellular activities by binding to proteins and nucleic acids. As such, measuring MDA levels has became an important biochemical marker for detecting oxidative stress in various biological systems Morales & Munné-Bosch, 2019; Cordiano et al., 2023).

Oxidative stress can further be explained for what it is: it can be defined as a pathophysiologic condition brought about by the accumulation of reactive oxygen species above the level of its neutralization by body mechanisms (Tok et al., 2021). This can finally cause damage to lipids, proteins, and DNA due to higher concentrations of reactive oxygen species, hence acting as potential initiators for various neurodegenerative disorders (Gwozdzinski et al., 2021; Goodman & Bellen, 2022). If the situation of oxidative stress continues unabated, it can finally cause efforts taken by some body cells to protect against it via repair or apoptosis.

Antioxidants are biological compounds capable of inhibiting or reducing oxidative reactions. They are known to function in ways such as reducing or scavenging free radicals, inhibiting oxidative chain reactions, and reducing oxidative intermediates. Antioxidants therefore function as key inhibitors in preventing damage by oxidation (Chaudhary et al., 2023). If there is a substantial rise in intracellular levels of free radicals, which have highly reactive properties, they may inhibit the activity of natural protecting enzymes such as catalase, superoxide dismutase, and peroxidase. This may result in oxidative changes occurring in the lipid components of cellular membranes and cellular proteins, resulting in destructive and lethal cellular injury. Additionally, it is suggested that reactive oxygen species have a crucial part in regulating natural processes by impacting cellular signaling pathways (Iqbal et al., 2024).

ROS has a role in various physiological events through the stimulation of signaling in cellular proliferation and growth processes. However, increased ROS levels above the limit induce oxidative damage to nucleic acids, proteins, and lipids (Jomova et al., 2023). But ROS generation under physiological conditions is low and quickly degraded by the intrinsic antioxidant mechanisms of the cells. The key players in the antioxidant mechanism are the enzymes SOD, GPx, and CAT; although their location within the cells is different, the mechanism is collective (Gusti et al., 2021).

Oxidative stress and excessive ROS production have been considered to play a pivotal role in neurotoxicity mechanisms triggered by acetylcholinesterase inhibitors (AChEI). Brain cells are very vulnerable to oxidative stress. This may be explained by their high demand for both oxygen and energy consumption as well as their high lipid composition rich in polyunsaturated fatty acids peroxidation, as well as their poor antioxidant system (Khuanjing et al., 2021). In seizures caused by carbamate-based AChEIs, specifically those belonging to group AChEIs, ATP consumption per cell rises exponentially. In addition to this being reduced by oxidative phospholation inhibition, this undermines cellular energy homeostasis and antioxidant capabilities in leading to excessive ROS levels and neuronal damage (Nasrabadi et al., 2024).

Superoxide dismutases (SOD) are metal-based antioxidant enzymes and are the first defense mechanism against reactive oxygen species (ROS). Based on their metal components and sites of expression, there are three different isoforms one is found in mitochondria (Mn-SOD), another is cytoplasmic (Cu/Zn-SOD), and the last one is secreted outside and is also known as (EC-SOD). The enzymes are very important for shielding cells from the toxic powers of the superoxide anion radical in the presence of oxygen (Islam et al., 2024).

The SOD enzyme catalyzes the dismutation reaction of the superoxide radical according to a two-step mechanism, resulting in the production of hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). In the first step of this mechanism, the superoxide radical reduces a metal ion and an oxygen molecule is released. In the second step, the reduced metal ion is oxidized by the superoxide radical, and this results in the

production of hydrogen peroxide (Al-Faris et al., 2025).

The catalase enzyme is a very important component of the system offering protection against oxidative damage. The catalase enzyme catalyzes the decomposition of hydrogen peroxide produced inside a cell to produce innocuous products. The process is therefore another means through which oxidative stress is reduced. Under a situation where there is a higher concentration of H_2O_2 inside a particular cell, this catalase acts alongside other antioxidants and protects a particular cell from undergoing oxidative damages by converting hydrogen peroxide into water and oxygen (O_2) (Xu et al., 2022). In a scenario of low concentration of H_2O_2, it has also been observed that the affinity of the Glutathione peroxidase (GPx) enzyme to H_2O_2 is also high compared to the affinity of the Catalase enzyme (Nguyen et al., 2019).

Glutathione peroxidases (GPx) are some of the main antioxidant enzymes, which have an essential role in defending cells against the toxic action of reactive oxygen species (ROS) and are an effective barrier against free radicals. Glutathione peroxidases catalyze the reduction of hydrogen peroxide (H_2O_2) and various organic peroxides, employing the reducing agent glutathione (GSH) as an electron donor in the reaction. During the GPx reaction, GSH is oxidized to form glutathione disulfide (GSSG), an essential step in the maintenance of the redox balance of the cell. GPx enzymes help to safeguard the cell membrane and genetic information against any oxidation damage inflicted by ROS not only by reducing H_2O_2, but also other derivatives of organic peroxides like lipid hydroperoxide or DNA hydroperoxide (Čapek & Roušar, 2021).

There is limited literature available on the biological activities of agricultural by-products like waste onion skin extracts and secondary metabolites derived from them. Recently, researchers found that onion skin extract has a supportive role in relation to the wound healing process owing to its antibacterial activities (Mounir et al., 2023). The aim of conducting this study is to assess the effects of a combination of apple skin extract, which has been established for its antioxidant and anti-inflammatory biological activities, and onion dye on fibroblast cells, which have a crucial role in relation to wound healing. Within this context, it is intended to assess the wound healing activities of a combination on an in vitro wound model established in L929 fibroblast cells, as well as its effects in relation to the activities of superoxide dismutase (SOD) enzymes, catalase (CAT), and glutathione peroxidase (GPx), which belong to the intracellular antioxidant defense system of cells.

2. Materials and Methods

2.1. Extraction of Apple Peels

Apples were purchased from the local market. The apples were rinsed with de-ionized water and subsequently peeled with an autoclaved peeler. Dried samples of apple peels, obtained from drying the peels under ambient conditions and dried completely before being turned into a fine powder.Apple peel powder was kept in the dark at 4°C overnight prior to the extraction procedure.

The apple skins were ground into powder. The powder was extracted using 75% (v/v) methanol. The extract was then centrifuged twice at 5000 rpm for 15 minutes, and once at 10,000 rpm for 15 minutes. To prevent the oxidation of polyphenols, 2% (w/v) of ascorbic acid was used in the extraction process. The combined extract from centrifugation was then filtered to get the concentrated extract using a rotary evaporator at 40°C to eliminate methanol content. Lastly, 200 mL of deionized water was then added to the extract, and evaporation under vacuum was done to eliminate methanol fully.

The aqueous part, rich in polyphenols, was freeze-dried, and the dried extract was ground to a powder to get the apple peel extract (Tian et al., 2018; Kamdi et al., 2021).

2.2. Extraction Of Dye From Onion Skins

The onion skin used was that of red onion (*Allium cepa* L.). The skin was sourced from agricultural waste products. Initially, the onion skin was ground to enhance extraction quantity. The ground onion skin was therefore used for extraction, and 1500 mL of distilled water was added to it. In the first extraction process, boiling was conducted under gentle boiling conditions and a temperature of 120 °C for 15 minutes, followed by heating in an oven set at 80 °C for 4 hours. After the process of extraction was done, the samples were left to cool in room temperature and then filtered using 50 and 100 mesh sieves. This aqueous extract

was used as the dye from onion skin since it was acquired from onion peels (Keşmer et al., 2020).

2.3. Wound Healing (Scratch) Test

The L929 fibroblast cell line was placed in RPMI-1640 medium, pH 7.4, and seeded into 6-well culture plates at a concentration of 5×10^5 cells per well. Once the cells had attached to the surface of the culture well, they were allowed to grow to 80% confluence. This point was where a horizontal scratch or wound was made to the monolayer using a 10 µL sterile disposable pipette tip.

In order to remove the cellular debris, each well was washed twice with a volume of 2 mL of phosphate-buffered saline (PBS). Later, a mixture of onion skin dye and extracted appleskin dye was applied to the cells with concentrations measuring 15, 60, 125, and 250 µg/mL. Both negative controls and application groups with varying concentrations were used in the experiment.Closure rates of the wounds induced were assessed for 24, 48, and 72 hours using an inverted microscope. The microscopic images of the control and application groups were taken using ZEISS microscope and ZEN 3.1 software to analyze cell migration and percentage of cell closure at the wound site (Zhao et al., 2020).

2.4. Determination of Malondialdehyde Levels

Activities of enzymes were assayed by brand Bioassay Technology Laboratory ELISA kits. Cells at an appropriate density were seeded into culture flasks and incubated for 24 hours at an incubator with 5% CO_2. After this period, the application of apple peel extract to the cells at specified concentrations was done and thus experimental groups were formed. At the end of a two-night incubation period, the cells were detached from the flask surface by trypsin-EDTA and centrifuged for 15 minutes at 1500 rpm. The ELISA analyses were made to the obtained cell pellets in accordance with the kit manufacturer's protocol, and the absorbance values were read at a wavelength of 450 nm by a microplate reader(Mas-Bargues vd., 2021).

2.5. Determination of SOD, CAT, and GPx Enzyme Activities

Bioassay Technology Laboratory ELISA kits were employed to assess the functional capacity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzymes. The cells growing on the top surface of each flask when subsequent cells were at confluence were removed using trypsin-EDTA and then counted using a counter device, and then equal numbers were placed into each new flask and incubated for 24 hours inside an incubator with 5% CO_2.

Removal of the medium after incubation, subgrouping of cells into five different groups depending on the planned use: control, 15, 60, 125, and 250 µg/mL, and applying the apple skins and onion skins extract mixture to the cells at their predetermined respective concentrations and finally putting them back into the incubator. After completion of the time, the cells were washed using PBS, trypsinized using EDTA, and 10 µl of cells were collected in Eppendorf tubes. Cell analysis was done in each of the groups in turn. The samples were then centrifuged at 2000 rpm for 20 minutes to provide pellets of cells.

The ELISA test was carried out as described by the kit supplier. This started with the addition of each standard solution of concentrations 0, 1, 2, 3, 4, and 5 into each well of the 96-well plate at a volume of 50 µL. As per the protocol, 40 µL of samples from the application groups were placed in the wells, and 10 µL of the specific primer antibody was added. Later, 50 µL of streptavidin-HRP conjugate was added to the wells, and the plate was incubated at 37 °C for 60 minutes. The microtitre plates were then subjected to washing by washing buffer supplied in the kit, with five washes lasting 30 seconds each. This was followed by the addition of 50 µL solutions A and B, which was then incubated for 10 minutes at 37 °C. To arrest the reaction, a stop solution of 50 µL was added to each well, and finally, the readings of the absorbance value were taken at 450 nm by a microtitre plate reader (El-Bahr, 2015; Alici & Arabaci, 2016).

3. RESULTS

3.1. Evaluation of the Wound Healing Test

In the present study, treatments were made by incubation of L929 fibroblast cells with 15, 60, 125, and 250 µg/mL of a mixture of apple peel extract and onion peel dye for 24, 48, and 72 hours. The determination of the treatment groups versus the control group showed that cytotoxicity increased in parallel with the doses. Then, it was established that the concentrations at 15 and 60 µg/mL statistically significantly enhance fibroblast cell migration and wound

closure rate in both time frames, and the 60 µg/mL dose was determined as the dose which gives the best effect on wound closure. The highest rates of wound closure were at the 15 and 60 µg/mL concentrations, especially at the end of the 48-hour incubation time. Wound closure rate at 48 hours was observed to be significantly less in the 125 and 250 µg/mL dose groups compared to the rest of the doses. In addition, it was observed that the effect of all concentrations used on the cells after the 72-hour incubation period was cytotoxic. The migration abilities of the fibroblast cells treated with various concentrations are shown in Figure 1.

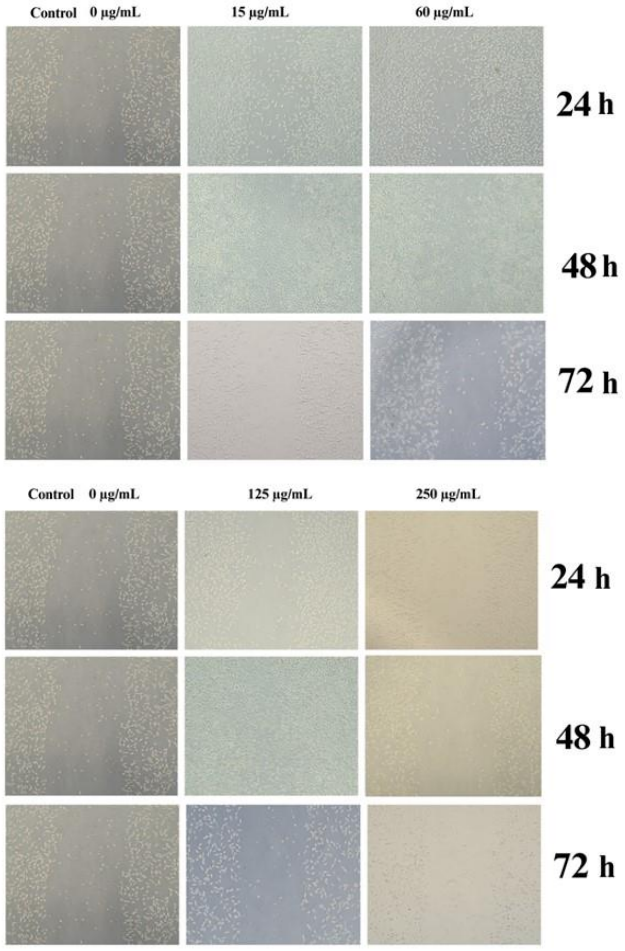

Figure 1. *The effects of L929 fibroblast cells on wound closure rates at 24, 48, and 72 hours.*

3.2. Evaluation Of Mda Levels

In evaluating the effect of the apple and onion peel extracts with gradually increasing concentrations on the level of malondialdehyde (MDA) in L929 fibroblast cells, it was found to have a significant effect in all groups in terms of MDA levels as compared to the control group ($P < 0.05$).

This shows that there was an induction of oxidative stress in the cells by both extracts, and the data was presented in Figure 2.

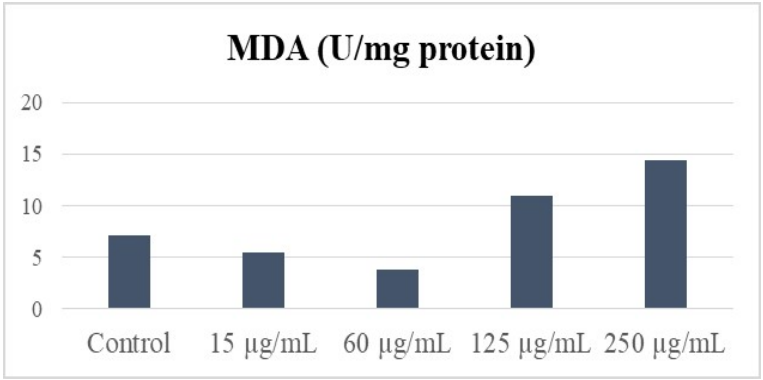

Figure 2. *Comparison of MDA Levels in the Control and Treatment Groups*

3.3. Evaluation of SOD Enzyme Activity

Enzyme activity of SOD was significantly different from the control when the treatment groups were compared to each other in light of increasing concentration of apple and onion peel extract; it decreased significantly in all the treatment groups compared to the control group ($P<0.05$). The results are presented in Figure 3.

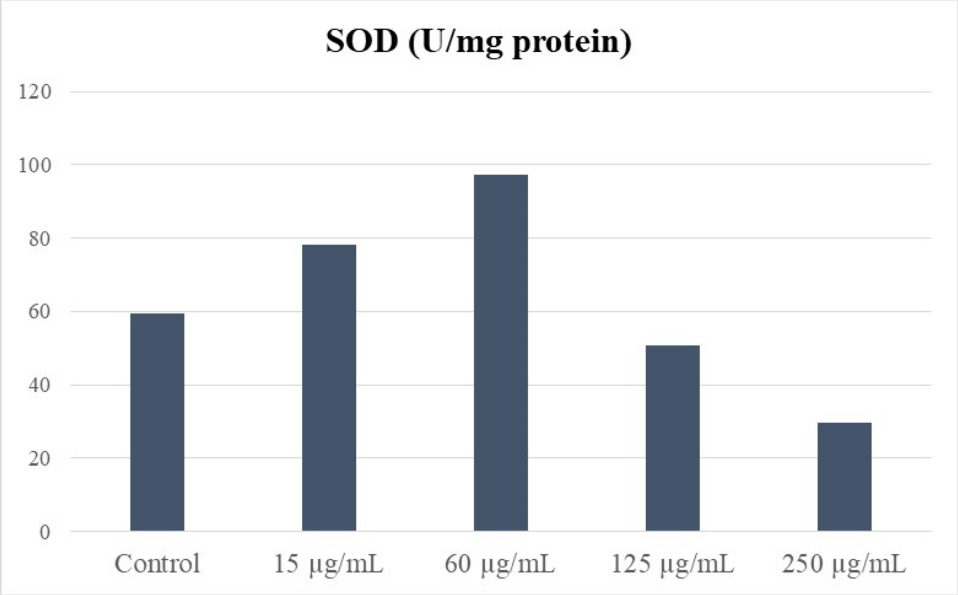

Figure 3. *Comparison of SOD Levels in the Control and Treatment Groups*

3.4. Evaluation of CAT Enzyme Activity

Comparing the CAT enzymes in the control group and the apple and onion peel extract treatment groups by increasing doses, it was observed that at a low and medium dose, the extract had a stimulating effect on antioxidant defense mechanisms at the cellular level compared to the control samples; however, at higher doses had an inhibitory action on antioxidant enzymes due to cytotoxic effects on cells.

The result shows a dose-dependent biphasic (hormetic) reaction of CAT enzymes ($P<0.05$). The obtained results are presented in Figure 4.

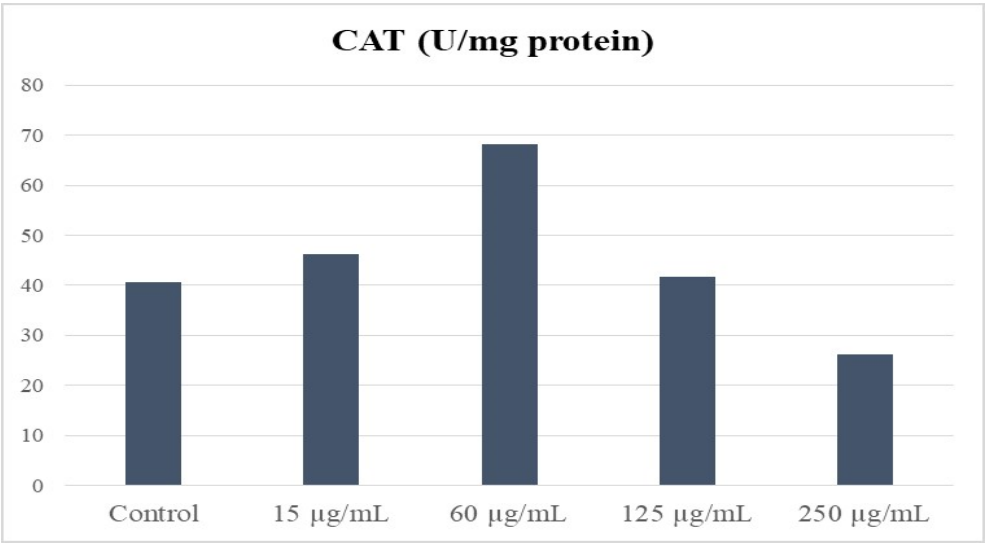

Figure 4. *Comparison of CAT Levels in the Control and Application Groups*

3.5. Evaluation of GPx Enzyme Activity

These results indicate that GPx enzyme activity is stimulated at low and medium concentrations, while its activity is suppressed at high doses, in agreement with the cellular stress and potential cytotoxic effects which might occur at such high extract doses. Overall, the findings indicate a dose-dependent, bidirectional effect of the extract on GPx enzyme activity ($P<0.05$). The findings are shown in Figure 5.

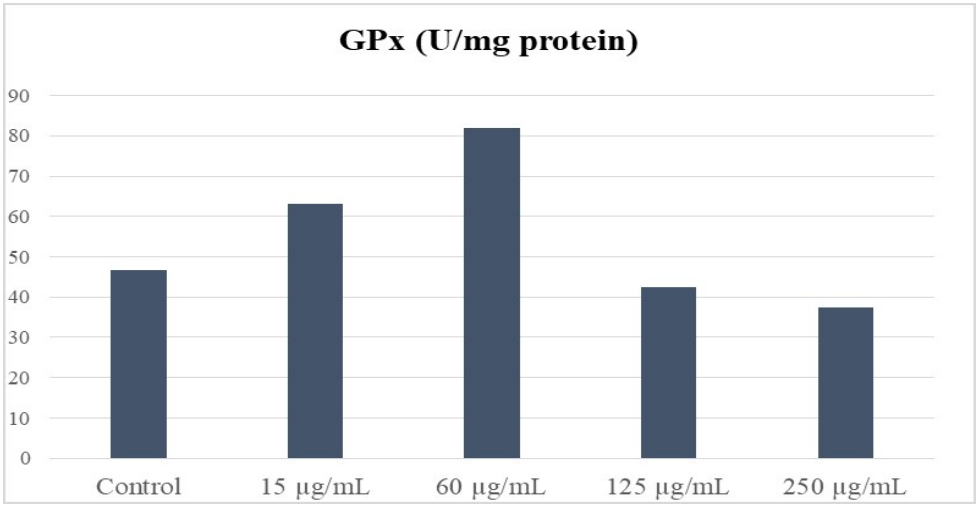

Figure 5. *Comparison of GPx Levels in the Control and Treatment Groups*

4. Conclusion and Discussion

Cell migration is considered one of the most important stages of the wound healing mechanism. The literature indicates that apple peel, with its rich phenolic content, particularly quercetin and catechin derivatives, supports cell proliferation. The protective effect of apple polyphenols on vascular endothelial cells and fibroblasts and their role in promoting migration have been emphasized. Similarly, studies on the efficacy of onion peel extracts (Allium cepa L.) in wound healing have shown that these natural products induce collagen synthesis and cell migration (Kim & Park, 2018). This experiment used L929 fibroblasts exposed to varied concentrations (15µg/mL, 60µg/mL, 125µg/mL, and 250µg/mL) of a mixture of apple peel extract and onion peel dye. The impact of these varied incubation periods (24 hours, 48 hours, and 72 hours) on the migration of cells was assessed. Analysis of the varied treatment groups showed significant cytotoxic effects against the control in increasing concentrations.

However, it was discovered that the concentrations of 15 µg/mL and 60 µg/mL statistically significantly promoted fibroblast cell migration and the rate of wound closure in both settings, and the concentration of 60 µg/mL was the most potent concentration in promoting wound closure. The highest rates of wound closure were recorded in the concentrations of 15 µg/mL and 60 µg/mL, especially after the 48-hour incubation period. However, the rates of wound closure were significantly lowered in the higher concentrations of 125 µg/mL and 250 µg/mL, and all concentrations were shown to cause cytotoxicity after the 72-hour incubation period. When evaluated in the context of antioxidant enzyme analysis results, compared to the control group, an increase in intracellular superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activity associated with wound healing was found in the low-dose groups (MDA) is associated with the pro-oxidant effects exhibited by plant extracts at high doses (Halliwell, 2014). In the high-dose groups, a decrease in antioxidant enzyme activity and an increase in MDA were observed, leading to higher peroxidation damage in membrane lipids compared to other groups and causing functional loss due to the maximum removal of intracellular reactive oxygen species (ROS).

The obtained data showed that a combination of apple peel and onion peel suppresses oxidative stress through the activation of the intracellular antioxidant defense system at appropriate concentrations, thus providing significant support for the wound healing process by inducing fibroblast cell migration via this mechanism. In this regard, it is thought that such a combination may offer a potential natural biotherapeutic agent that can be used in wound healing.

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