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Effect of EVOO's polyphenol on reduced tissue damage due to electrical burns in mouse models

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ABSTRACT
Background:Burn injury have significant morbidity and mortality rates. Tissue around burn injury frequently damaged as a secondary wound (secondary thermal injury). There is no clear consensus of second degree burns treatment, as many variability of wound care materials. Glutathione is one of the antioxidants that can minimize the effects of oxidative stress by increasing intracellular Glutathione and accelerating wound healing by increasing the capacity of fibroblast contraction.
Aim: The study seeks to examine the effect of topical Glutathione on the healing process of deep dermal burns using male Wistar rats (*Rattus Novergicus*) as the experimental subjects.
Material and Methods: This research is a true experimental study using post test only control group design. Thirty-two rats were treated for burns on the backs (dorsum) by attaching heated iron plate at 100°C. The samples were divided into 2 groups of 16 rats in treated group with topical Glutathione application, and 16 rats in control group with topical placebo. The area of epithelialization and microscopic observation of fibroblasts, collagen and neovascularization was carried out on 5th day (D-5) and 12th day (D-12) studies.
Results:Obtained in microscopic increased fibroblasts maturation, collagen deposition, neovascularization and macroscopic re-epithelialization in group of rat samples applied by topical Glutathione in D-5 and D-12 studies and obtained significant differences compared with rat groups that were applied topical placebo.
Conclusion: Topical Glutathione can accelerate wound healing of deep dermal burns in male rats (Rattus Novergicus) wistar strain.

Keywords:Burn Injury, Burns, Deep dermal, Glutathione, Rats wistar strain (*Rattus Novergicus*)

1. Introduction

Electrical burns frequently occur and cause severe tissue damage. The severity depends on the intensity and type of current, electrical load, injury location, contact duration, and secondary injuries(Bailey, 1989).Early medical treatment can reduce damage, infection risk, muscle necrosis, cardiac arrest, or arrhythmia (Solem et al., 1977). In the United States, electrical injuries cause 1000 deaths annually and 3000 hospitalizations. About 20% occur in children, particularly teenagers and toddlers, mostly at home(Lee, 1997).

Electrical injuries can damage muscles and bones in addition to skin and soft tissues, occurring in 3-5% of thermal burn patients(Kumar et al., 2013). High serum creatine kinase levels are linked to severe muscle damage(Abdulloh & Ni'mah, 2023) and can serve as a prognostic marker(Kopp et al., 2004).The main cause of severe tissue necrosis in electrical injuries is microvascular circulation disorders. Large cells like muscles and nerve cells are more vulnerable to electrical damage, and an increase in serum creatine kinase (CK) is closely related to cell damage and muscle disorders, indicating muscle necrosis(Brancaccio et al., 2007).

Current treatments focus more on symptoms than preventing tissue damage. Polyphenols in EVOO have antioxidant and anti-inflammatory effects, aiding the healing of electrical burns and reducing morbidity and mortality(Suha et al., 2016). These polyphenols stabilize free radicals, reduce inflammatory mediator production, and modulate kinase enzyme activation involved in the inflammation process.

This background encourages researchers to study the effects of polyphenols in EVOO on tissue damage prevention and healing in electrical burn injuries. The systemic effects of polyphenols in controlling kinase enzymes in the blood play a preventive role against hepatotoxicity and nephrotoxicity. The administration of antioxidant polyphenols in EVOO is expected to influence tissue healing in electrical burns, thereby reducing morbidity and mortality rates in burn patients.

2. Methodology

Study Design

This study utilized an experimental design, specifically a randomized post-test only control group design. The subjects were male Wistar rats (Rattus norvegicus), approximately 3 months old and weighing 250-300 grams. Electrical burns were induced by attaching electrodes around the left upper and right lower extremities, followed by cardiac aspiration to collect blood samples. The rats were housed in polypropylene cages (30 cm x 40 cm x 15 cm) with six rats per cage, maintained in the same room with a constant temperature of around 24°C. The cages were ventilated, covered with wire mesh, and had bedding of rice husk. The rats were given one week to adapt before the experiment began. They were fed Par-G pellet feed at a rate of 20 grams per day per rat and provided with ad libitum access to bottled drinking water (Aqua brand). After the study, the rats were sacrificed, decapitated, and cremated to ensure proper disposal. This research conducted from January until May 2024. The research is conducted at the Biochemistry Laboratory of the Faculty of Medicine, Universitas Airlangga, Surabaya, with blood specimen analysis performed at the Surabaya Health Laboratory Center.

Electric Wound Rat Model

The model rat with electric burn wounds was created by delivering electrical energy to rats that had been anesthetized by intraperitoneal injection of Ketalar (ketamine hydrochloride) 15 mg (100 mg/kg) diluted with saline solution. The anesthetized rats were positioned supine on an applicator table. Prior to attaching electrodes, metal plates measuring 1 cm wide surrounded the upper left and lower right extremities, and shaving and application of ultrasound gel as a conductive material on the skin were performed. Electrical current of 220 V AC was applied to these extremities for 10 seconds at 450–500 mA. The current was measured using an avometer.

Polyphenols in Extra Virgin Olive Oil (EVOO)

This study uses Bertolli brand Extra Virgin Olive Oil (EVOO), original type, as the source

of polyphenols, which has USDA Organic certification. The dosage administered will be 0.125-0.15 mL/day in a 1 mL syringe formulation. The dosage selection has been adjusted based on previous research findings(González-Correa et al., 2007).

Measurement of the area of necrotic skin tissue

The necrotic zone is assessed macroscopically, judging from its bluish, gray to black coloration, with distinct borders. It is then measured using a template (transparent mica) interpolated into Visitrak®, yielding the area of the necrotic zone in cm^2. This measurement is taken for all sample subjects.

Measurement of serum creatine kinase levels

Measurement of serum creatine kinase levels involves obtaining a 3 mL blood specimen via cardiac puncture, which is then collected in an EDTA tube and stored at 4-10°C. The specimen is processed within 6 hours post-collection. The assay will utilize a Creatinine Assay Kit and measurements will be taken at 540 nm absorbance using a spectrophotometer. This blood specimen analysis will be conducted at the Surabaya Health Laboratory Center.

Data analysis

In this study, the necrosis wound area and creatine kinase levels were measured using an independent t-test. Results were considered significant if $p<0.05$. Statistical analysis was conducted using SPSS version 25.

3. ResultsandDiscussion

Analysis

The analysis of the administration of polyphenols in EVOO on the necrotic skin area in electrical burn injuries is presented in Figure 1.

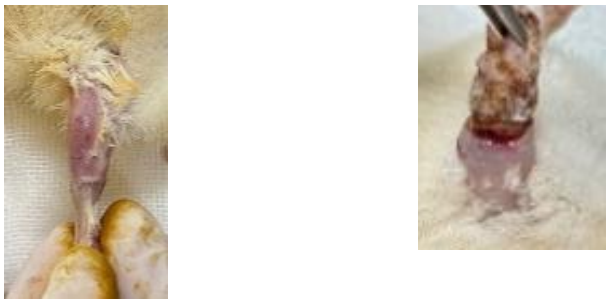

Figure 1. Necrotic Area of Electric Wound Model

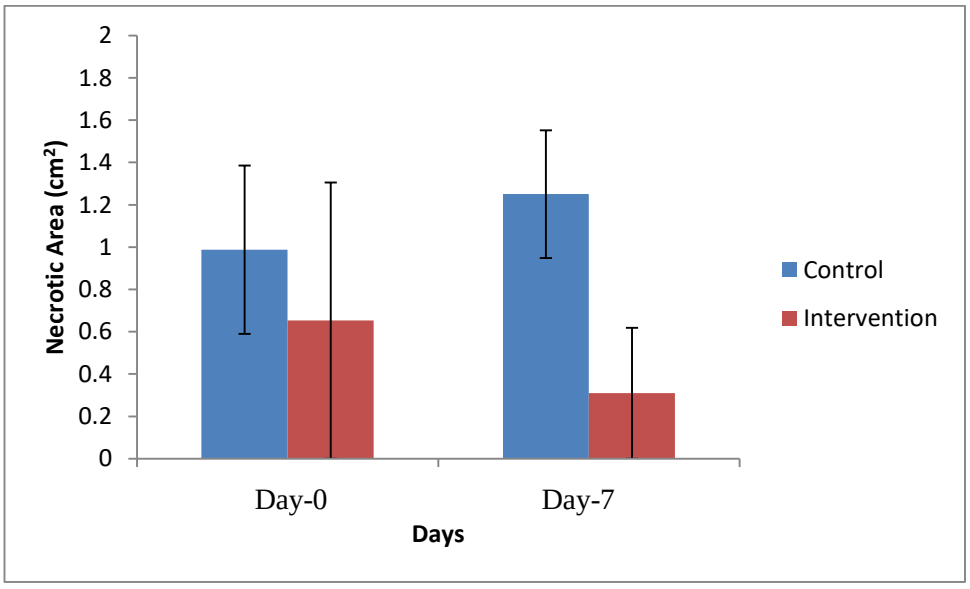

Figure 2. Necrotic Area

In Figure 2 showed the average necrotic area on day 0 was 0.98 cm² with a standard deviation of 0.39, and on day 7 it was 1.25 cm² with a standard deviation of 0.30. For the treatment group, the average on day 0 was 0.65 cm² with a standard deviation of 0.65, and on day 7 it was 0.31 cm² with a standard deviation of 0.31. The p-value for the comparison on day 0 was 0.056, indicating no significant difference, whereas the p-value for day 7 was 0.001, indicating a significant difference after treatment.

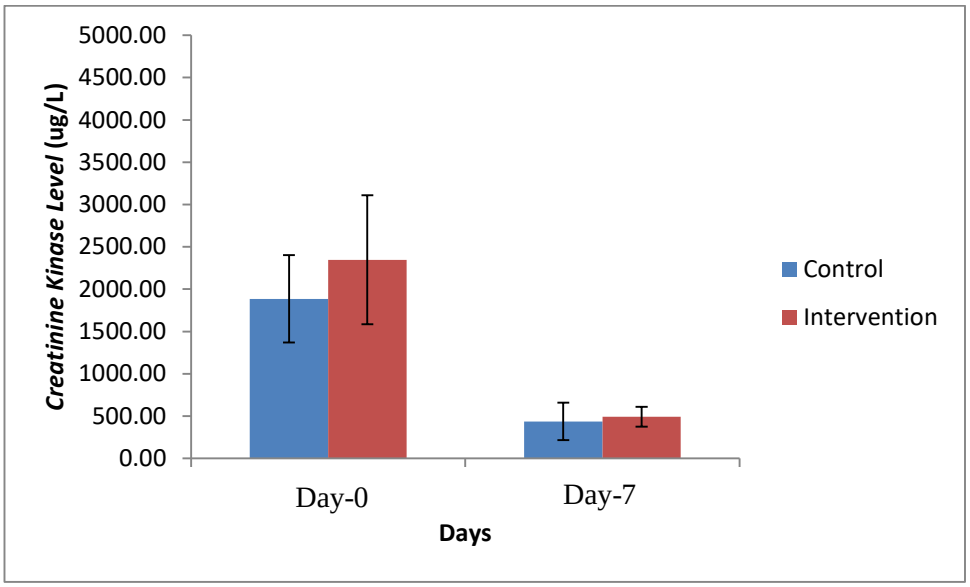

Figure 3. Creatinine Kinase Level

Figure 3 showed the average of creatine kinase levels. On day 0, the control group had an average level of 1885.25 U/L with a standard deviation of 516.40, and the treatment group had an average of 2346.88 U/L with a standard deviation of 762.03. On day 7, the control group had an average level of 435.75 U/L with a standard deviation of 221.00, while the treatment group had an average of 492.13 U/L with a standard deviation of 117.74. The p-value for day 0 is 0.178, indicating that the difference between the two groups on day 0 is not significant. On day 7, the p-value is 0.535, also indicating no significant difference.

Discussion

The Effect of Polyphenols in EVOO on Skin Necrosis Area in Electrical Burn Injuries

This study demonstrates that the administration of polyphenols in extra virgin olive oil (EVOO) reduces the area of skin necrosis in electrical burns in Wistar rats. In this study, the electrical current was applied using a 220 V AC current for 10 seconds at a current of 450–500 mA. When the electrical current passes through the body, the skin, which serves as the initial point of contact, can suffer extensive damage. The degree of skin damage is influenced by the current voltage, skin resistance, and duration of contact. Electrical current can cause direct burns to the skin through the thermal energy generated by the skin's resistance to the passing current. Additionally, electrical energy can disrupt the integrity of cell membranes in the skin through electroporation, leading to cell death. Subcutaneous vascular damage may also occur, resulting in thrombosis, tissue ischemia, and necrosis(Marsden, 2019).

The antioxidant activity mechanism of polyphenols in EVOO involves direct absorption of free radicals through hydrogen atom transfer (HAT) or single electron transfer (SET) mechanisms, as well as transition metal chelation (TMC)(Bhuyan & Handique, 2022). Polyphenols in EVOO protect cells from pro-oxidant damage by modulating cellular antioxidant status. This effect is observed in the restoration of altered glutathione levels and various antioxidant enzyme activities caused by oxidative stress, such as glutathione reductase, peroxidase, S-transferase, and catalase, thereby reducing lipid peroxidation(Illam et al., 2017). This antioxidant mechanism reduces reactive oxygen species (ROS), thereby accelerating tissue regeneration(Shafiq et al., 2021).

The findings of this study are supported by previous research showing that certain types of polyphenols have been proven to reduce necrosis. For instance, Hu et al. (2024) evaluated the administration of resveratrol, a type of polyphenol, on experimentally wounded rat skin. Their results indicated that this polyphenol stimulated cell growth, reduced cell death, and accelerated skin repair and collagen production in burn wounds(Hu et al., 2024).Furthermore, Orlowski et al. (2020) found that polyphenol-modified nanoparticles could reduce necrosis in wounds without causing local irritation or inflammation(Orlowski et al., 2020). Another study by Chen et al. (2020) showed that polyphenol compounds from green tea could reduce necrosis in diabetic rats by regulating the PI3K/AKT signaling pathway(Chen et al., 2020). Similarly, Zhao et al. (2019) found that polyphenolic extracts from longan seeds could reduce necrosis in second-degree burn wounds by enhancing new dermal tissue formation and capillary blood vessel formation(Zhao et al., 2019). The study by Van de Velde et al. (2019) also indicated that polyphenol extracts from strawberries have anti-inflammatory effects and promote the migration and proliferation of skin fibroblasts(Van de Velde et al., 2019).

Additionally, research evaluating the oral consumption of olive oil has shown that it can reduce necrosis in burn wounds in patients with burns over certain body surface areas(Najmi et al., 2015).Another study evaluating various substances, including seabuckthorn, olive oil, and their mixtures on full-thickness burn wounds in rats, found that the groups treated with seabuckthorn, olive oil, or their mixtures experienced faster wound contraction compared to the control group(Edraki et al., 2014).

The Effect of Polyphenols in EVOO on Creatine Kinase Levels in Electrical Burn Injuries

This study demonstrates that the administration of polyphenols in extra virgin olive oil (EVOO) does not affect creatine kinase (CK) levels. CK is an enzyme found in muscle tissue, and its serum levels can increase following muscle injury. In the context of burn injuries, elevated CK levels are generally associated with muscle damage due to electrical burns and deep thermal burns(Stevenson et al., 2008).

In this study, the decrease in CK levels in both the treatment and control groups did not show a significant difference. These results are consistent with previous research indicating that CK levels do not correlate with the extent of high-voltage electrical burns(Kopp et al., 2004).However, other studies have shown that CK levels increase following low-voltage electrical induction in humans(Pallett et al., 2013).

The administration of polyphenols in EVOO did not result in a significant reduction in CK levels compared to the control group. This may be due to serum creatinine not being a

specific marker for muscle damage. The increased CK levels in the study rats may also be attributed to myocardial damage caused by electrical induction(Balestrino, 2021).Additionally, the rats given the intervention were more active, which could lead to increased CK levels. This suggests that the decrease in CK levels does not align with the improvement in burn wounds in rats. Moreover, changes in CK levels are influenced by the activity levels of the rats, potentially introducing bias during sample collection (Kim & Wierzbicki, 2021). EVOO also contains several active phenolic compounds such as hydroxytyrosol, tyrosol, oleuropein, and ligstroside, which may interfere with the effectiveness of the polyphenols(Rodríguez-López et al., 2020).

This study shows that the administration of polyphenols from olive oil reduces the area of electrical burn wounds but does not affect CK levels. The study has several limitations. First, it does not evaluate the side effects of polyphenol use. Second, it is conducted on rat models, necessitating further research on higher mammalian models. Third, fixation of the rats is required to avoid affecting CK levels.

4. Conclusion

Based on the results and discussion above, it can be concluded that the administration of polyphenols effectively reduces the area of skin necrosis in electrical burn injuries in a rat model. Additionally, it has been demonstrated that polyphenols decrease blood creatine kinase levels in these injuries by day 7. These findings highlight the potential therapeutic benefits of polyphenols in managing and treating electrical burn injuries, emphasizing their role in mitigating tissue damage and improving recovery outcomes.

Further research is needed in several areas. First, oral administration of polyphenols should be investigated over a longer duration and with additional supporting variables to assess the potential side effects of polyphenol use in treating electrical burn injuries. Second, the use of polyphenols to reduce necrosis should be tested in higher mammals to determine its efficacy and safety, potentially paving the way for its use as an adjunct therapy in electrical burn treatment. Third, comprehensive studies on the side effects of polyphenols are essential to ensure their safety in medical applications. Lastly, studies involving the fixation of rats during experiments are necessary to minimize bias and accurately evaluate the impact of polyphenols on creatine kinase levels and wound healing.

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Conflicts of Interest

The Authors declares that there is no conflict of interest.

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