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Assessment of the Beneficial Role of Propolis in Oral Wound Healing

Aseel Kamil Hameed[1], Alaa Abdulkhaliq Hussein[2], Alaa Hussein Saadon[2]
[1]College of Dentistry, University of Basrah, Iraq
[2]College of Veterinary, University of Basrah, Iraq

Abstract

This work examined the therapeutic potential of propolis gel as a natural topical agent to enhance wound healing in the hard-palatine region of local adult male rabbits (*Oryctolagus cuniculus*). Forty-eight rabbits were randomized into eight groups (four controls, C3, C7, C14 and C21; four treatment groups: T3, T7, T14 and T21). All animals performed a 10x 3 mm longitudinal incision in the hard palate at the same distance. Treatment groups received a single film of prepared propolis gel once daily for 3, 7, 14, or 21 days, while the control (C) group received no topical application. Bloods were collected in intervals for biochemical analyses. Biochemical assays confirmed the findings, demonstrating a highly significant decrease in NO and MDA ($p \leq 0.01–0.0001$) and a highly significant increase in CAT and SOD ($p \leq 0.01–0.0001$), implying lower oxidative stress as compared to the control groups. Topical application of propolis gel has beneficial effects that are attributed to anti-inflammatory, antioxidant, antimicrobial, and proangiogenic activities, promoting collagen deposition, tissue repair, and re-epithelialization. Propolis may represent a safe, cost-effective approach for reducing healing time and morbidity associated with oral wounds.

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Email: aseel.hameed@uobasrah.edu.iq

Introduction

The oral cavity is composed of several functioning units, such as the hard palate, gingiva, tongue, and buccal mucosa, that together perform important functions like mastication, speech production, taste sensation, and the initial process of digestion; the hard palate, as a firm separation between the oral and nasal cavities, is important for swallowing and articulation. The result is that the mucosal surface of the oral cavity is constantly exposed to mechanical force (due to mastication), thermal fluctuation (as food and beverages enter the oral cavity), as well as a variety of bacteria, fungi, and viruses [1]. Accordingly, this area is highly susceptible to a variety of types of lesions, such as trauma wounds, burns, ulcers, and surgical defects. Injury of these tissues frequently causes a considerable amount of pain, disturbance in mandibular function and a high incidence of infection [2].

Traditional treatment for injuries of hard palate and oral mucosa is to remove or eliminate the cause, clean up the mouth, and use dropwise medicine application from antibacterial agents to antiseptic drugs, apply one drop at a time into injury site. Furthermore, chlorhexidine mouth rinses, corticosteroids and different types of antibiotics can be used. Nevertheless, tissue irritation, the increasing risk of microbial resistance to antibiotics drugs; unsatisfactory continuous regeneration effects and caused potential side-effects to have encouraged researchers to investigate alternative treatments that are both safe and biocompatible material effectively promoting the regenerated tissue not only for its antimicrobial protective capability [3].

One of the most interesting natural candidates is propolis which is a resinous substance processed by honeybees from botanical exudates. This substance is known for its flavonoids, polyphenolic compounds and aromatic acids that provides antimicrobial, anti-inflammatory, antioxidant and wound-healing properties [4]. In an experimental model in laboratory animals of excisional palatal wounds, propolis was reported to favor greater contraction, healing and less inflammation than chlorhexidine-treated or nontreated wound [5]. Comparable results have been reported with a buccal mucosa wounds model, which was treated with propolis that enhanced the proliferation of fibroblasts and epithelial cells as well as upregulated vimentin expression, while it significantly decreased inflammatory cell penetration [6].

Propolis also has been documented in clinical studies to have therapeutic efficacy. In a randomized, double-blind split-mouth study, postoperative application of 5% propolis ointment to the palatal donor sites after FGGA reduced pain and advanced healing significantly compared with no intervention in control sites [7]. Equally, in head and neck cancer patients receiving radiotherapy, better efficacy was reported when using a propolis formula mouthwash along with shorter duration of mucositis healing and improved mucosa repair; almost 100% recovery was achieved in most treated patients within one week without remarkable adverse events [8]. Direct evidence on hard palate trauma does need to be determined, however a systematic review and Meta-analysis of RAS found both topical and systemic propolis were effective treatments for lessening pain, erythema and duration of healing when compared with placebo, but the quality of evidence was rated low [4].

In addition to its accelerating effects on the reduction of damaged tissue, propolis has proven strong anti-biofilm activities and inhibits growth of major oral pathogens Such as: Streptococcus mutans, Candida albicans and Staphylococcus aureus [3]. Taken together, these results highlight the potential of propolis in accelerating wound healing and of its activity in re-epithelialization, inflammation modulation, and antimicrobial inhibition. Therefore, propolis becomes a potential candidate for adjunct or substitute therapy of injuries in the oral mucosa and hard palate.

The objective of this study is to investigate the extent to which local propolis can be used as a healing accelerator of oral and hard palate wounds. It enumerates the bio-therapeutic effects of propolis, that might replace drugs used in oral cavity lesions by clinical dentistry to reduce side-effects, interactions, and contra-indications. Having the potential to be used as a natural and economical remedy for oral mucosa lesions, propolis could play a role in shortening the healing period and decreasing post-injury complications.

This aim will be achieved through the following objectives, including examining the effectiveness and safety of propolis to reduce healing time compared with a placebo, and investigating the antioxidant capacity of propolis in speeding up the process of wound healing.

Materials and Methodos

The extraction was designed for purification of major propolis bioactives, especially flavonoids, phenolic acids and their esters, wax and beeswax impurities being discarded. To maintain stability, the raw propolis was initially frozen at −20 °C. Fragments of the frozen material were cut, chopped into small pieces, defrosted and finally ground with an electric pulverizer (National, Japan). The extraction procedure was derived from previous work [9,10]. A flask beaker and the solution stored in a dark place for 14 days with continuous stirring by a magnetic stirrer using (100 g of powdered propolis: 1000 mL of 96% ethanol) [11]. The mixture was filtered initially through sterile gauze to remove the larger particles and subsequently filtrated at Whatman No. 1 filter paper to give the wax-free ethanolic extract.

The clarified filtrate was transferred into sterile glass Petri dishes and left to evaporate at room temperature until complete dryness. The resulting residue was carefully scraped off and stored at 4 °C until required for experimental use.

It was prepared with carboxy polymethylene (Carbopol 934) and propylene glycol [12], in which 2 grams of Carbopol 934 were dispersed in 50 ml of distilled water with continuous stirring; then 2 ml of propylene glycol and 25 grams of propolis extract were added, and the volume was made up to 100 ml by adding the remaining distilled water.

The ingredients were mixed well with stirring, and then triethanolamine was added dropwise to the formulation to adjust the pH (6.5-6.8), obtaining the gel at the required concentration. An identical procedure was used to obtain a control sample without propolis addition [1].

This investigation was carried out on 48 adult male indigenous domestic rabbits (Oryctolagus cuniculus), weighing 1.9-2 kg. The animals were divided into two equal groups: T (treated) and C (control). For each group, it was then divided into four equally sized subgroups (n = 6) and kept under specific pathogen-free conditions (12/12 h light/dark cycle at 25 °C with a proper humidity), ensuring the availability of water and commercial rabbit feed. The subgroups were divided as follows:

C3 group: 10 × 3 mm longitudinal hard palate incisions were made on each of the six rabbits, which never received any treatment other than daily topical application with propolis-free gel for 3 days.

T3 group: 10 × 3 mm longitudinal hard palate incisions were made on each of the six rabbits, and they were treated with propolis-containing gel daily for 3 days.

C7 group: 10 × 3 mm longitudinal hard palate incisions were made on each of the six rabbits, and they received daily treatment with propolis-free gel for 7 days.

T7 group: 10 × 3 mm longitudinal hard palate incisions were made on each of the six rabbits, and they received daily treatment with propolis gel for 7 days.

C14 group: 10 × 3 mm longitudinal hard palate incisions were made on each of the six rabbits, and they received daily treatment with propolis-free gel for 14 days.

T14 group: 10 × 3 mm longitudinal hard palate incisions were made on each of the six rabbits, and they received daily treatment with propolis gel for 14 days.

C21 group: 10 × 3 mm longitudinal hard palate incisions were made on each of the six rabbits, and they received daily treatment with propolis-free gel for 21 days.

T21 group: 10 × 3-mm hard palate linear incisions were made on each of the six rabbits, followed by daily application of propolis gel for a period of 21 days.

On day 0 and under aseptic conditions, sterile surgical wounds measuring 3 × 10 mm were longitudinally made in the oral hard palate of all animals using established rabbit oral wound protocols with minor modifications [13-15]. Anesthesia was administered intramuscularly with a mixture of 35 mg/kg ketamine and 5 mg/kg xylazine prior to surgery, which is the normal anesthetic protocol for short surgical duration in rabbits [16]. After wound creation, treated subgroups received daily topical applications of a thin film of propolis-containing gel for their respective durations, whereas control subgroups received propolis-free gel similarly. After each treatment period, 10 mL of blood was collected into tubes without anticoagulant for biochemical assays [17].

Serum samples were stored at −20°C and thawed on ice immediately before analysis to preserve the integrity of the biomarkers, and indirect measurements of nitric oxide were made by assaying for its metabolite, nitrite. The mixture of serum and Griess reagent (sulfanilamide in H3PO4, N-(1-naphthyl) ethylenediamine dihydrochloride (NEED), 26.7 mol/L HCl) was incubated at room temperature for 10 min followed by measuring the absorbance at 540 nm to determine nitrite concentration using a standard curve prepared with NaNO2 [18].

The activity of catalase was determined in a spectrophotometer by measuring the decomposition of hydrogen peroxide. The 1 ml assay mixture consisted of phosphate buffer (pH = 7.0), hydrogen peroxide with a serum sample, reduction in absorbance at 240 nm was recorded for a minute at room temperature, and catalase activity was measured as units/mg protein, one unit being the amount of enzyme to decompose 1 µmol of H2O2/minute) [19].

SOD activity was measured by the SOD, which inhibited NBT reduction in a xanthine-xanthine oxidase system. The reaction mixture consisted of phosphate buffer (pH 7.8), NBT, xanthine, x anthinex oxidase and serum sample. Solution absorbance was read at 560 nm every 5 min at room temperature. 1 Unit of

SOD was defined as the amount that caused 50% inhibition in NBT reduction [20].

Levels of MDA, an index of lipid peroxidation, were estimated by the thiobarbituric acid reactive substances (TBARS) test. Serum was mixed with thiobarbituric acid and acetic acid and heated in a boiling water bath for 1h. The samples were then cooled and centrifuged. The absorbance of the supernatant was determined at 532 nm. The contents of MDA were determined based on 1,1,3,3- tetramethoxypropane standard curve [21].

Statistical analysis was carried out using IBM SPSS Statistics version 27.0 program. Group differences were analyzed using one way ANOVA followed by Tukey's post hoc comparisons. A P-value of <0.05 was considered statistically significant [22-24].

Results

The current antioxidant biomarkers studied are Nitric Oxide (NO), Catalase (CAT), Superoxide Dismutase (SOD), and Malondialdehyde (MDA).

Nitric oxide (NO) is a vasoactive mediator used to analyze and promote the relaxation of blood vessels during wound healing. It is linked with inflammation to differentiate between worsening wounds and progressing healing; therefore, the current results showed that there is a significant ($p \leq 0.01$) decrease in levels of NO in the T3 group (9.17±0.56) when compared to the C3 group (11.05±0.56), as in Figure 1. Besides, the results of NO showed highly significant ($p \leq 0.0001$) decreases in the T7 group (9.63±0.56), T14 group (8.84±0.56), and T21 group (8.01±0.56) when compared to the C7 group (13.66±0.56), C14 group (14.22±0.56), and C21 group (16.26±0.56), respectively.

Catalase (CAT) is an effective inhibitor of reactive oxygen species (ROS) that mediates to prolong the process of wound healing, therefore, the current results on catalase (CAT) showed a non-significant ($p \geq 0.01$) differences between the T3 group (5.33±0.42) and T7 group (5.87±0.426) when compared to C3 group (4.81±0.42) and C7 group (4.63±0.426), respectively (Figure 2). Moreover, the results of catalase (CAT) showed highly significant ($p \leq 0.001$) and ($p \leq 0.0001$) increases in its levels in the T14 group (6.24±0.42) and T21 group (6.76±0.426) when compared to its levels in the C14 group (4.01±0.42) and C21 group (3.97±0.426), respectively.

Superoxide dismutase (SOD) is the crucial oxidative stress biomarker that can identify the disorders that occurs in the wound healing steps; therefore, the current results of SOD showed a non-significant ($p \geq 0.01$) differences between the T3 group (609.9±28.9) and T7 group (642.2±28.91) when compared to C3 group (522.2±28.9) and C7 group (502.9±28.91), respectively (Figure 3).

On the other hand, the results of SOD showed highly significant ($p \leq 0.0001$) increases in its levels in the T14 (642.2±28.91) group and T21 group (649.5±28.9) when compared to its levels in the C14 group (470.2±28.91) and C21 group (454.5±28.9), respectively.

Malondialdehyde (MDA) is used as a biomarker to assess oxidative stress in the wound healing phases; therefore, the current results of MDA showed a significant ($p \leq 0.01$) decrease in the T3 group (44.01±4.33) and T7 group (50.28±4.338) when compared to C3 group (57.92±4.33) and C7 group (65.56±4.338), respectively; besides, a highly significant ($p \leq 0.0001$) decrease in T14 group (46.43±4.33), and T21 group (39.76±4.33) when compared to its level in the C14 group (72.57±4.33) and C21 group (75.51±4.33), respectively (Figure 4).

Discussion

Biochemical analysis is instrumental in determining any deterioration at the level of cellular and organic injuries; thus, the current study analyzed the main antioxidant biomarkers to give an idea of how regeneration is processed.

Flavonoids, polyphenols, and phenolic acids—of which their esters are thought to be the primary contributors—are the primary sources of propolis's antioxidant qualities [25]. Due to their capacity to scavenge oxidative agents and lessen the production of ROS, flavonoids have been studied for their antioxidant activity [26].

Nitric oxide (NO) levels significantly decreased in the propolis-treated group when compared to the control group. This is because NO disrupts the regeneration process, which in turn causes oxidative stress, which damages DNA and disrupts cellular energy metabolism by reducing the function of cellular mitochondria and calcium homeostasis, which co-occurred to varying degrees [27,28]. Increased NO production occurs in every aberrant physiological state because it disrupts the metabolic pathways in the mitochondria. In comparison, glutathione cellular depletion can result from elevated NO levels.

In addition, the propolis has an antioxidative activity against elevated NO levels since it exhibited a significant decrease in NO levels in propolis-treated animals [29]. The antioxidant profile of propolis was also done by Marzouk et al., (2007), who investigated the anti-inflammatory effect of propolis, in which the propolis plays an important role in the inhibition of NO production [30].

Catalase is a very common, helpful enzyme that is present in all organisms that are exposed to oxygen. The enzyme catalyzes the disintegration of harmful cellular H_2O_2 into water and oxygen; Catalase is an extremely efficient enzyme with the ability to break down millions of H_2O_2 molecules into water and oxygen per second [31]. The current study showed that the catalase was highly significantly increased in the late stages of healing at T14 and T21 when compared to C14 and C21 because the catalase enzyme in propolis-treated groups was concentrated at a high level to reduce the oxidation of ROS in the hard palate wound since the catalase is one contributor of propolis and bee honey, also known as increased oxidative stress lead to loss of antioxidants such as SOD, catalase in the non-propolis group [32].

Previous work [33] consistent with our study showed that propolis has a potential protection role in pulmonary oxidative stress by increasing the catalase activity to protect the lungs from hyperoxia; because propolis has strong antioxidant and scavenging abilities, therefore native propolis might be used as a strong plant-based antioxidant effective not only in physiological conditions but also in cases that require prolonged high concentration of oxygen.

The current study on the role of superoxide dismutase enzyme SOD was analyzed to compare its role among the propolis-treated group and control in which it showed a highly significant increase in the propolis-treated group in the late stages of wound healing compared to the control, this because the propolis has antioxidant activity via its contents like flavonoids, polyphenol group, and antioxidant vitamins, minerals, caffeic acid, phenethyl ester, terpenoid, steroid, that have a significant role in healing acceleration and elevate the SOD activity to reduce ROS; since the SOD accelerate the dismutation of the toxic superoxide radicals that produced during oxidative stress into H_2O_2 and oxygen; and then the catalase hydrolyzing H_2O_2 into water.

Another research done on the role of propolis as an antioxidant supplement in animals [34] agreed with our results on propolis when augmenting the natural cellular antioxidants like catalase and SOD which showed that the propolis used as an antioxidant in broilers exposed to lead showed similar antioxidant effects as vitamin C in the case of oxidative stress. Also, it showed that the activities of SOD and CAT increased depending on the cellular defense mechanisms under oxidative stress by the increased MDA level; moreover, it reported an increase in SOD and CAT activity in the state of lipid peroxidation [15,35,36].

The current study on the MDA level showed a sharp increase in control wounding compared to the propolis-treated group at all regeneration intervals since the ROS produced by the damaged cells as well as the invaded inflammatory cells that defend the infection of the

wounded tissues to clear the lytic cells in non-propolis treated group; while the MDA showed significantly decreased in all stages of wound healing in the propolis treated group because the propolis has an active ingredient acts as antioxidant agents like flavonoids and other constitution which lead to diminishing the ROS and other oxidative stress factors, therefore, the MDA is being dropped since it is formed as the byproduct of oxidative stress reaction which reflects the oxidative status of the individuals.

Delayed duration of the process of wound healing produces a higher number of damaged cells accompanied by inflammation and ROS generation, produced by damaged cells and immune cells; these reflected the increased level of plasma MDA [37]. It was mentioned that the level of MDA increased over time post-wound injury in the laboratory animals that didn't use the propolis supplement [15,38].

El-Guendouz, et al., (2019) reported that the propolis exhibits antiradical activity since it reduces the plasma and tissue MDA levels and erythrocyte and tissue SOD and CAT activities [39]. Besides, it was documented that the administration of propolis significantly decreased MDA in rat liver tissue, in which the propolis phenolic components and their antioxidant activity may be responsible for this effect [23,24,40]. Also shown propolis administration to rats led to restoring the inflammatory markers, which showed a significant reduction in oxidative damage caused by oxidative stress, it is considered a very important protective effect against hepatotoxicity [23,24,41].

Conclusion

Topical application of propolis gel has beneficial effects that are attributed to anti-inflammatory, antioxidant, antimicrobial, and proangiogenic activities, promoting collagen deposition, tissue repair, and re-epithelialization. Propolis may represent a safe, cost-effective approach for reducing healing time and morbidity associated with oral wounds.

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Conflict of Interest

The authors declare no conflict of interest.

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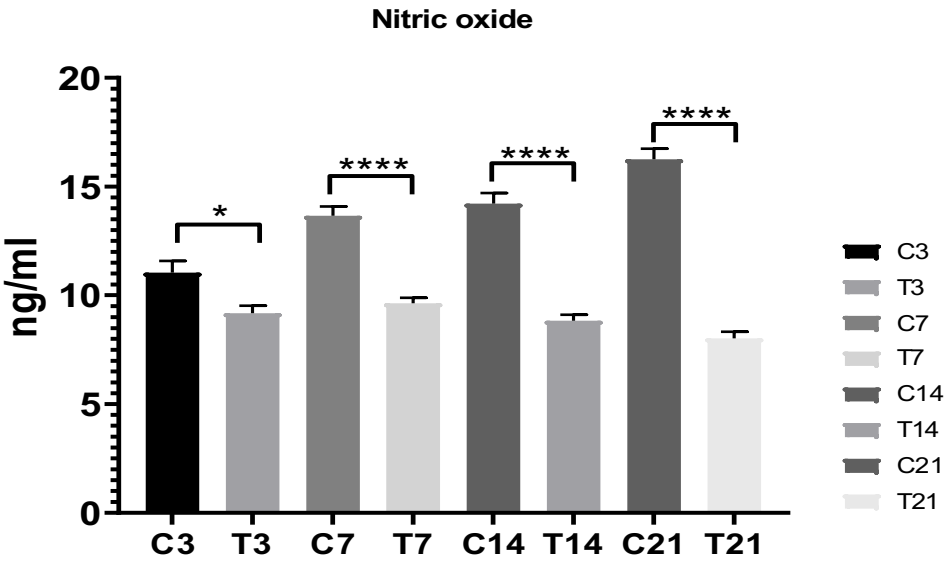

Figure 1. The biochemical level of nitric oxide (NO) biomarker among treated groups (T3, T7, T14, and T21) and control groups (C3, C7, C14, and C21). The symbol (****) refers to a highly significant difference between treated groups and control groups at level (P≤0.0001); whereas the symbol (*) refers to a significant difference between treated groups and control groups at level (P≤0.01); the measurement unit is ng\ml.

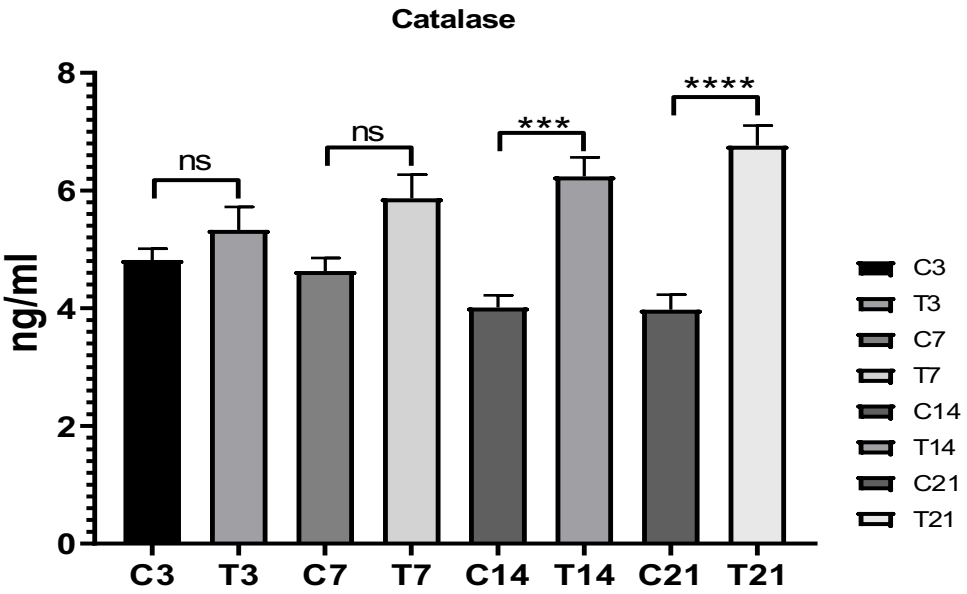

Figure 2. The biochemical level of catalase (CAT) biomarker among treated groups (T3, T7, T14, and T21) and control groups (C3, C7, C14, and C21). The symbol (***) and (****) refers to a highly significant difference between treated groups and control groups at levels (P≤0.001) and (P≤0.0001); while the symbol (ns) refers to a non-significant difference between treated groups and control groups at level (P≥0.01); the measurement unit is ng\ml.

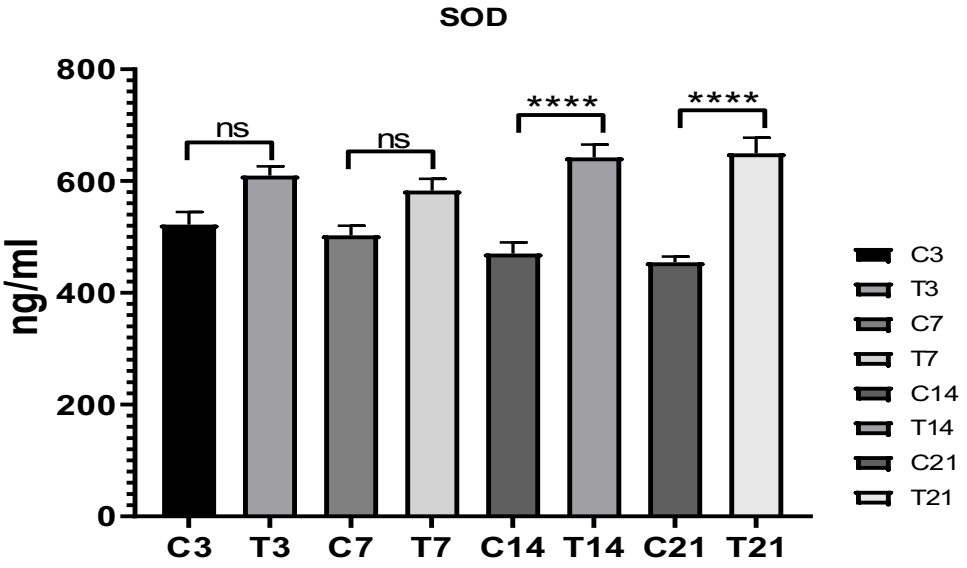

Figure 3. The biochemical level of superoxide dismutase (SOD) biomarker among treated groups (T3, T7, T14, and T21) and control groups (C3, C7, C14, and C21). The symbol (****) refers to a highly significant difference between treated groups and control groups at level (P≤0.0001); while the symbol (ns) refers to a non-significant difference between treated groups and control groups at level (P≥0.01); the measurement unit is ng\ml.

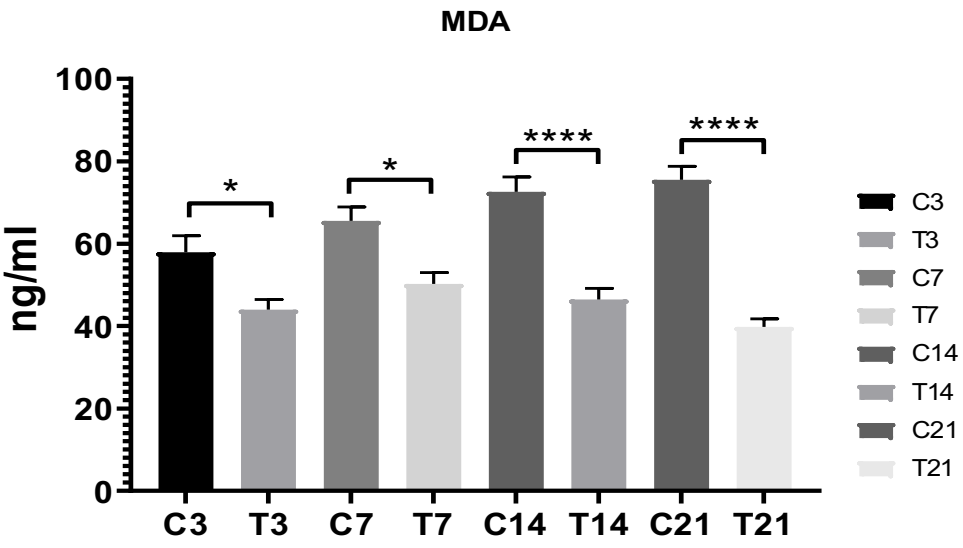

Figure 4. The biochemical level of Malondialdehyde (MDA) biomarker among treated groups (T3, T7, T14, and T21) and control groups (C3, C7, C14, and C21). The symbol (****) refers to a highly significant difference between treated groups and control groups at level (P≤0.0001); whereas the symbol (*) refers to a significant difference between treated groups and control groups at level (P≤0.01); the measurement unit is ng\ml.