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# Radiographic evaluation to assess bone density upon administration of Low-Intensity 660 nm Laser Therapy alongside Nano-hydroxyapatite and antioxidant compounds (N-acetylcysteine) for Dental Socket Preservation Following Extraction Utilizing CBCT

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**ABSTRACT**:

**Purpose**: To assess the preservation of bone density upon tooth extraction utilising low-level laser therapy together with nano-hydroxyapatite and antioxidant substance (N-acetylcysteine).
**Materials and Methods**: The study sample comprised 30 randomly selected patients, evenly distributed across three groups. Group I, the socket was sealed by an absorbable collagen membrane without graft materials following tooth extraction. In Group II, a combination of nano-hydroxyapatite and antioxidant agents (N-acetylcysteine) was introduced into the empty socket, followed by placing a bioabsorbable collagenous membrane over the grafting material, then sutured. Group III, the same mixture of nano-hydroxyapatite and antioxidant agents was applied to the extracted socket, followed by the adaptation of an absorbable collagen membrane over the graft and suturing, in addition to low-level 660 nm laser therapy (LLLT) administered three times weekly for two weeks, power of 25 mW, and duration of 120 seconds. Bone density at the extraction site was evaluated using cone beam computed tomography (CBCT). Bone density was measured immediately and at three and six months postoperatively.
**Results:** The NHA and NAC groups subjected to LLLT exhibited the most significant improvement in bone density measurements compared to the other groups.
**Conclusions**: a combination of NHA and antioxidant agents (N-acetylcysteine), along with the application of LLLT to alveolar socket post-extraction, minimized bone resorption after six months from grafting that can be safely utilized for socket maintenance.

**Keywords**: LLLT, bone healing, NHA, NAC

## I. NTRODUCTION

To prevent changes in hard and soft tissues caused by tooth loss, alveolar socket preservation involves inserting biomaterials into the extracted tooth's socket during extraction [1].
Alloplastic, allogeneic, xenogeneic, and Autogenous bone substitutes, along with membranous barriers due to their capacity to preserve space, facilitate rapid bone turnover, and exhibit biocompatibility, as well as additional compounds as bone morphogenetic protein (BMP), platelet-rich plasma, platelet-rich fibrin (PRF), and titanium-prepared platelet-rich fibrin (T-PRF), have all been concurrently documented with various other substances [2-8].

Hydroxyapatite (HA), the predominant component of the mineral composition of bone and teeth and exhibiting biocompatibility, has been extensively studied as a biomaterial within the medical field [9].
Its significant similarity to the natural bone composition largely explains the regenerative potential mechanism of NHA [10, 11]. Furthermore, bone tissue promptly adheres to hydroxyapatite, resulting in the deposition of newly formed bone [12]. The HA surface has demonstrated the ability to enhance osteoblastic cell proliferation, differentiation, and adhesion [13]. In addition to its antioxidant properties, N-acetylcysteine (NAC) demonstrates a range of pharmacological effects on

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osteoblast lineage cells [14]. NAC proved to enhance bone regeneration on implanted biomaterial by improving biomaterial cytocompatibility and reducing wound infection [15-17]. NAC has been shown to enhance osteogenesis by promoting differentiation of osteoblast-like cells rather than inducing bone marrow-derived mesenchymal stem cells (BMSCs) to differentiate into osteoblast progenitor cells [18].

Diode lasers employed in Low-level laser therapy (LLLT) have gained popularity for promoting faster wound healing. The photobiomodulation theory, or biostimulation, underpins its mechanism of action. Due to its nonthermal influence, it exerts a biostimulatory effect that facilitates modifications in cellular behavior. It induces cellular modifications by influencing membrane calcium channels and the mitochondrial respiratory chain. This intervention promotes cellular metabolism and proliferation, thereby facilitating expedited wound healing. Furthermore, LLLT exerts an immediate analgesic effect [19].

Photobiomodulation therapy has been demonstrated to expedite and augment the formation of new bone tissue in animals in vivo, enhance epithelial cell proliferation, and foster angiogenesis [20-25]. Diode lasers emitting light at 904 and 660 nm influence bone healing and enhance osteoblastic activity [8, 26-27].

## II. MATERIALS AND INSTRUMENTS

### *A- METHODOLOGY*

Following the execution of an informed consent form detailing the procedure and expected outcomes post-treatment, a careful selection of thirty patients from the Oral and Maxillofacial Surgery Department's Outpatient Clinic Department of Kobri el Kobba Military Hospital were divided into three groups based on treatment methods for participation in this study.

**Inclusion criteria:**

- Medically healthy adult patients aged 20–40 years.
- A hopeless, decayed root or teeth where extraction is the recommended treatment.
- posterior mandibular teeth.

**Exclusion criteria:**

- Systemically diseased participants.
- Existence of acute infections.
- Heavily smoking patients.
- Pregnancy or nursing.

**Grouping**:

To enhance the reliability of our findings. Thirty patients were randomized into three treatment groups, & each group was made up of ten individuals:

1. Group I: Following tooth extraction, the socket was covered with an absorbable collagen membrane (COLLAFLEX®, UNI CARE BIOMEDICAL, USA) and sutured with Vicryl 4-0 (Ethicon, USA).
2. Group II: After the tooth was extracted, the Socket was filled using a combination of Nano-hydroxyapatite (IngeniOs HA, USA) plus antioxidant agents (N-acetylcysteine) (Acetylcysteine, Pharmazell GmbH, Rosenheimer Str. 4383064 Raubling, Germany). Then, the socket was sutured using a resorbable membrane.
3. Group III: After the tooth was extracted, the Socket was filled using a combination of Nano-hydroxyapatite plus antioxidant agents (N-acetylcysteine). Then, the socket was sutured using a resorbable membrane, and low-level laser therapy (LLLT) was administered.

**Surgical Procedures:**

L.A. (Scandonest 3%, Canada) was achieved based on group diversity. The severely decayed tooth was extracted as a traumatically as possible to prevent soft tissue damage and bone loss. Following the dental extraction in the first group, a membrane was applied over the socket, and Vicryl 4-0 sutures were utilized.

In the second group, an empty alveolus received a Nano-hydroxyapatite filling plus antioxidant (N-acetylcysteine), after which the graft was covered with a collagen membrane that was then sutured [Figure 1].

**Figure 1:** alveolar socket after grafting with NHA & NAC & suturing

In the third group, the socket was preserved with a combination of Nano-hydroxyapatite and antioxidant agents (N-acetylcysteine). A bioabsorbable collagenous membrane was positioned, covering the graft and sutured in place. 660 nm LLLT was conducted three times weekly for two weeks using the Sirolaser advance plus (Dentsply Sirona, GMBH, Germany) at a power of 25 mW and for 120 seconds on the buccal, lingual, and occlusal surfaces [Figure 2].

**Figure 2:** Sirolaser advance plus

**Radiographic Evaluation:**

CBCT was conducted immediately post-surgery (baseline), at 3 months, and at 6 months to assess bone density as follows:
Bone density measurements were conducted precisely at the socket's Centre, allowing for a thorough comparison across multiple time intervals. This approach ensures a comprehensive understanding of changes over time. [Figure 3].

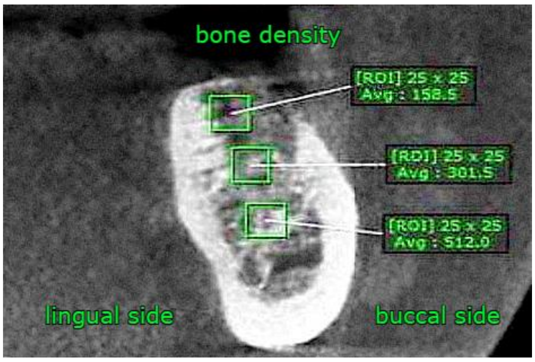


**Figure 3:** Coronal view Cone Beam CT showing bone density measurement

## III. Results

**Statistical analysis:**

IBM SPSS (version 20.0) was used for data analysis.

**Demographic data:**

The age range of the thirty patients was as follows: Study Group I ranged from 21.0 to 39.0 years, with a mean age of 31.20 ± 5.75 years; Research Group II also ranged from 21.0 to 39.0 years, with a mean age of 31.20 ± 5.75 years; and Study Group III ranged from 21.0 to 39.0 years, with a mean age of 29.61 ± 5.53 years. The average age of the groups did not exhibit a statistically significant difference. Control Group I comprised five males and five females, Study Group II comprised five males and five females, and Study Group III included four males and six females. The sex distributions of the groups did not exhibit statistically significant differences.

**Radiographic evaluation (bone density):**

The average bone density remained the same in all groups for six months. Upon comparing the bone density among the three groups at each follow-up interval, it was noted that group III exhibited the highest values, except at baseline, where group II held the highest value, while group I consistently recorded the lowest values at all follow-up times. The bone density increased in group III after the six-month interval, but the increase was nonsignificant.

*Table 1*: Overall mean bone density immediately, at 3 months, and 6 months post-extraction, along with the significance levels of the differences observed.

| **Group** | **Group I (control) N=10** | **Group II (study) N=10** | **Group III (study) N=10** | **H** | **P** |
|---|---|---|---|---|---|
| **Bone density** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | | |
| Base line | 386.95 ± 130.35 | 418.64 ± 165.67 | 405.24 ± 180.07 | 0.173 | 0.917 (ns) |
| 3 months | 427.12 ± 128.91a | 480.54 ± 171.36 | 489.47 ± 179.16 | 1.18 | 0.555 (ns) |
| 6 months | 482.06 ± 136.36a | 544.40 ± 150.81 | 606.61 ± 178.04 | 1.75 | 0.416 (ns) |

H: test of Kruskal–Wallis.
P: p-value for comparison between the studied groups.
*: "significance level $p \leq 0.05$; ns=non-significant".

## IV. DISCUSSION

To optimize outcomes, surgeons must ensure high-quality bone structure and appropriate dimensions for implant insertion by preserving the alveolar ridge [28].
Various grafting materials have been utilized for socket preservation, including autogenous bone grafts, allografts, xenografts, and alloplasts [29].
Takata et al. [30] assert that incorporating collagen and HA may Encourage the formation of new bone. during the healing process & establish a more conducive medium for osteoblastic cell proliferation after adhesion and differentiation.
Supplementary HA markedly enhanced the mineralization of newly formed bone in vivo, as reported by Rajzer et al. [31]
A study on mouse calvarial cell cultures suggests that NAC may enhance osteoblastic development.[32]
This study utilized a combination of NHA and NAC as a bone substitute, with and without LLLT at 660 nm, 25 mW for 120 seconds, applied to the buccal, lingual, and occlusal surfaces three times weekly for two weeks, to assess the improvement of bone formation and density following tooth extraction.
Meanwhile, the NHA and NAC groups exhibited more enhancement in bone density than the control group.but non significantly.
The application of lasers increased the quantity of viable osteocytes in the irradiated area, demonstrating the substantial potential for new bone formation.
Other studies indicate that LLLT positively influences osteoblast development and proliferation in cell cultures and animal models of bone formation. [36, 37]
A recent study employing Nano-hydroxyapatite bone grafts and NAC to maintain the alveolar ridge post-tooth extraction. The minimal width reduction was observed in nanohydroxyapatite combined with NAC and NAC alone. The most significant enhancement in bone density and the

minimal decrease in bone height were noted with Nano-hydroxyapatite combined with NAC and NAC independently [38].

Brawn et al. investigated the impact of LED phototherapy on a sinus augmented with a granular bovine bony substitute in a distinct clinical case study [39]. A 20 mW/cm$^2$ Light Emitting Diode (LED) phototherapy treatment was administered twice daily for ten minutes over two weeks. A histological examination of a biopsy conducted four weeks later indicated that LED phototherapy had enhanced recovery.

F. Mafra de Lima et al. assert that LLLT and NAC synergistically augment MIP-2 (Macrophage Inflammatory Protein 2) mRNA expression in alveolar macrophages under the influence of lipopolysaccharide or $H_2O_2$. They propose that intracellular reactive oxygen species generation and NF-kB signaling may be implicated [40].

**Limitations**

The duration of observation in this study may be limited.

## IV-CONCLUSION

Under the constraints of the present study, LLLT combined with a mixture of nanohydroxyapatite (NHA) and antioxidant agents (N-acetylcysteine) as a graft in the alveolar socket can be effectively employed for ridge preservation following tooth extraction.

**Recommendations**

Future studies may necessitate histological assessments.

Additional research is required to examine the effects of NHA and NAC over extended observation periods (12 to 24 months).

**Declarations**

**Funding:**

The authors financed this research independently.

**Data availability:**

All data pertinent to this research were gathered from the participants utilizing CBCT, which facilitated recording their bone density measurements post-treatment.

**Ethics approval:**

Ethical approval reference: NILES-EC-CU 24/10/16. Additionally, consent was documented in a written format that complied with the institutional ethics committee's approval and was comprehended by each participant.

**Competing interests:**

There are no conflicts of interest.

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