https://doi.org/10.52973/rcfcv-e35677
Revista Científica, FCV-LUZ / Vol. XXXV

Role of Algerian propolis on hematobiochemical parameters and bone consolidation mechanisms in Rabbits receiving autoclaved diaphyseal allografts

Rol del propóleo argelino en los parámetros hematobioquímicos y los mecanismos de consolidación ósea en conejos que reciben injertos diafisarios autoclavados

Abdellatif Boudra[1], Rachid Merati[1], Abdelkader Berrani[2], Wassim Yezli[2], Laredj Hocine[3], Mourad Benbeguara[3], Esma Zineddine[4]*

[1]*University of Tiaret, Laboratory of Hygiene and Animal Pathology. 14000, Tiaret, Algeria.*
[2]*University of Tiaret, Faculty of Natural and Life Sciences. 14000, Tiaret, Algeria.*
[3]*University of Tiaret, Laboratory of Agro–Biotechnology and Nutrition in Semi–Arid Areas, Faculty of Natural and Life Sciences. 14000, Tiaret, Algeria.*
[4]*University of Djillali Liabès, Laboratory of Nutrition, Pathology, Agro–Biotechnology and Health. Sidi Bel Abbès, Algeria.*
Corresponding author: abdellatif.boudra@univ–tiaret.dz

ABSTRACT

This study investigated the effects of Algerian propolis extract from Laghouat on cortical allograft integration in New Zealand rabbits. Fifty adult male rabbits (mean weight: 2.5 kg) were used, including 20 donors. The 30 recipients were divided into two groups (n = 15 each), further split into five subgroups of three. The control group received an autoclaved cortical allograft, fixed with a pin and reinforced with a metal suture. The experimental group underwent the same procedure, but the allograft was coated with a thin layer of ethanolic extract of Laghouat Algerian propolis (EEPLA) during surgery. Blood samples were collected preoperatively and at 3, 7, 10, and 12 days post–surgery, as well as at 12 weeks.Hematobiochemical analysis revealed significant findings. The propolis–coated allograft group (ACWP) exhibited a marked decrease in leukocyte levels ($P<0.01$) and highly significant variations in thrombocyte levels ($P<0.001$). Alkaline phosphatase activity increased significantly ($P<0.001$) in the ACWP group, suggesting enhanced bone metabolism. No significant changes in calcium levels were observed in either group, but phosphorus levels increased significantly ($P<0.001$) in the control group (AWP) post–surgery.These findings suggest that EEPLA exerts an immunomodulatory effect and contains anti–platelet aggregation compounds. Additionally, preoperative blood loss led to a post–surgical decrease in hemoglobin levels. The elevated alkaline phosphatase activity in the AWP group indicates a potential osteoinductive role of EEPLA, promoting osteoblast formation. Furthermore, the stabilization of phosphorus levels in the ACWP group suggests that Laghouat propolis may help mitigate phosphorus deficiency, contributing to improved bone healing.

Key words: Propolis extract; bone graft; alkaline phosphatase; osteoinduction; hematobiochemical analysis

RESUMEN

Este estudio investigó los efectos del extracto de propóleo argelino de Laghouat en la integración de aloinjertos corticales en conejos de Nueva Zelanda. Se utilizaron cincuenta conejos machos adultos (peso medio: 2,5 kg), incluidos 20 donantes. Los 30 receptores se dividieron en dos grupos (n = 15 cada uno), subdivididos en cinco subgrupos de tres. El grupo control recibió un aloinjerto cortical autoclavado, fijado con un pin y reforzado con una sutura metálica. El grupo experimental se sometió al mismo procedimiento, pero el aloinjerto fue recubierto con una fina capa de extracto etanólico de propóleo argelino de Laghouat (EEPLA) durante la cirugía. Se tomaron muestras de sangre antes de la cirugía y a los 3, 7, 10 y 12 días después de la operación, así como a las 12 semanas. El análisis hematobioquímico reveló hallazgos significativos. El grupo con aloinjerto recubierto de propóleo (ACWP) mostró una disminución marcada en los niveles de leucocitos ($P<0,01$) y variaciones altamente significativas en los niveles de trombocitos ($P<0,001$). La actividad de la fosfatasa alcalina aumentó significativamente ($P<0,001$) en el grupo ACWP, lo que sugiere una mejora en el metabolismo óseo. No se observaron cambios significativos en los niveles de calcio en ninguno de los grupos, pero los niveles de fósforo aumentaron significativamente ($P<0,001$) en el grupo control (AWP) después de la cirugía. Estos resultados sugieren que el EEPLA ejerce un efecto inmunomodulador y contiene compuestos antiagregantes plaquetarios. Además, la pérdida de sangre preoperatoria condujo a una disminución de los niveles de hemoglobina después de la cirugía. El aumento de la actividad de la fosfatasa alcalina en el grupo AWP indica un posible papel osteoinductor del EEPLA, favoreciendo la formación de osteoblastos. Además, la estabilización de los niveles de fósforo en el grupo ACWP sugiere que el propóleo de Laghouat podría ayudar a mitigar la deficiencia de fósforo, contribuyendo a la mejora de la cicatrización ósea.

Palabras clave: Extracto de propóleo; injerto óseo; fosfatasa alcalina; osteoinducción; análisis hematobioquímico

Received: 15/04/2025 Accepted: 28/06/2025 Published: 16/07/2025

INTRODUCTION

Allografting is a transplantation technique involving the transfer of tissue from a donor to a recipient of the same species but with different genetic codes [1]. Allografts can be used in fragmented or structural forms and may be cortical, cancellous, or cortico–cancellous [2]. Due to the limitations associated with autograft harvesting, allografts have become a widely employed alternative in clinical and experimental research [3]. Bone grafting can be used as an implanted material to promote bone healing, either independently or in combination with other substances. Most of the research on the effects of propolis polyphenols on bone comes from in vitro and in vivo studies [2, 4].

Recent studies have shown that applying a thin layer of propolis to autoclaved diaphyseal bone allografts enhances rapid callus formation at the interfaces and may also help prevent bone loss [5, 6, 7].

Propolis, a resinous substance collected by honey bees, is mixed with wax to construct hives. It has a distinctive aroma and ranges in color from yellow to dark brown. Commercial propolis extracts are available in aqueous, ethanol, glycol, and oil–based forms [8]. Propolis contains over 300 chemical compounds, including flavonoids, phenolic acids, enzymes (such as glucose oxidase and catalase), vitamins, organic acids, free amino acids, and proteins [9]. The composition of propolis varies based on its geographic origin, reflecting the diversity of wild plants in different regions. Its biological properties are believed to result from the synergistic effects of its bioactive components [7]. Furthermore, the safety of propolis and its bioactive components has been validated through extensive research in both humans and animals [10].

The aim of this study is to demonstrate the therapeutic benefits of Algerian propolis from the south in enhancing the consolidation of bone allografts through hematobiochemical monitoring.

MATERIALS AND METHODS

Propolis extraction and composition

According to the methods outlined by Lahouel *et al.;* Hendi *et al.;* Pereira *et al.* [11, 12, 13], 20 g (Analytical balance. Kern – max 120 mg, Germany) of raw Propolis from southern Algeria, Laghouat Province was mixed with 200 mL of 70% ethanol (SCIALCHIM–Ethanol 96%–Lot N SZBG1810H, Algeria) in flasks shielded from light. The mixture was stirred periodically using a magnetic stirrer (IKA electromagnetic stirrer, Germany) in a dark environment. The ethanolic solution was then filtered through Whatman filter paper No. 1. The filtrate was transferred to petri dishes, and the ethanol was evaporated in an oven (Heraeus intrument – fabric number :95111970, Germany) set to 45°C. The purified propolis extract was then scraped off and stored in a dark place at 4°C (Condor, Algeria) for future use. Boudra *et al.* [14] reported that the raw propolis from this region contained 99.80 mg of total polyphenols, expressed as gallic acid equivalents per gram, and 54.50 mg of total flavonoids, expressed as quercetin equivalents per gram.

Biological material and surgical intervention

Cortical allografts were harvested from the diaphysis of the left femurs of male New Zealand rabbits *(Oryctolagus cuniculus).* The grafts were directly wrapped in autoclave paper (SteriClean, ISO 11607, REF 230112, Germany) and hermetically sealed using an impulse sealer (Type PFS-300, China). The prepared grafts were then sterilized by autoclaving at 134°C for 15 min (Webeco GmbH, Type A, No. 919995, Germany).

The surgical procedure began with premedication consisting of a double injection of Acepromazine(0.75 mg·kg^{-1}, intramuscularly) and Xylazine (2.5 mg·kg^{-1}, subcutaneously). Anesthesia was induced and maintained with ketamine administered intramuscularly at a dose of 35 mg·kg^{-1}.

The study included fifty adult male New Zealand rabbits, each weighing an average of 2.5 kg. Twenty of them were used as donors. The remaining 30 recipient rabbits were divided into two groups of 15, each further subdivided into five subgroups of three animals.

- The first group (control) received cortical allografts that had been autoclaved, fixed with a simple intramedullary pin(Kirschner, Grermany), and reinforced with a metallic suture(Stainless steel wire – BRINS 0.40 mm – 067804242, France).
- The second group underwent the same procedure, except that the cortical allografts were coated with a thin layer of propolis (62 mg at a concentration of 100 mg·mL^{-1}) [7, 15, 16, 17, 18, 19] (FIGS. 1A and 1B).

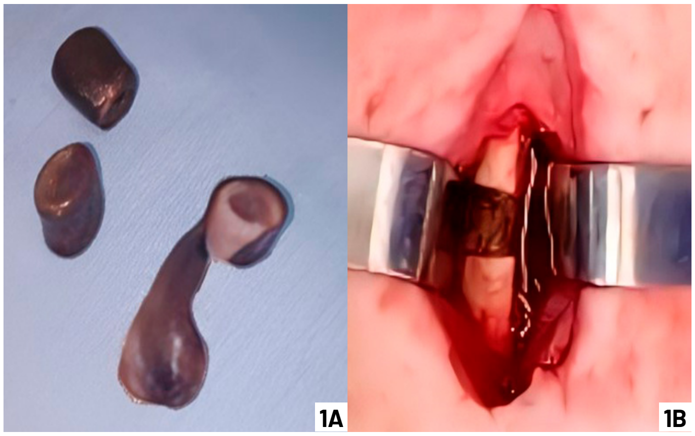

FIGURE 1. A: EEPLA (Ethanolic extract of Laghouat Algerian propolis) and Allogeneicgraft. B: Allogeneic graft covered with EEPLA (Ethanolic extract of Laghouat Algerian propolis)

Hematobiochemical monitoring:

In accordance with the biological events outlined in TABLES I and II, blood samples were collected from each animal before and after the surgical procedure, at the end of each biological event (3, 7, 10, and 12 days (d), and more than 12 weeks or 3 months), as indicated in TABLES I and II.

TABLE I
Distribution of groups according to biological events

AWP														
AWP3			**AWP7**			**AWP10**			**AWP12**			**AWP+12**		
R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
ACWP														
ACWP3			**ACWP7**			**ACWP10**			**ACWP12**			**ACWP+12**		
R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3

AWP: Allograft Without Propolis 3, 7, 10, 12, +12(90) days post–surgery. ACWP: Allograft Covered With Propolis 3, 7, 10, 12, +12(90) days post–surgery. R: Rabbits (1/2/3)

TABLE II
Classification of biological events observed during the implantation of osteoinductive protein

Days	0-3	4-7	8-10	10-12	+12 (3Months)
Biological Events	Proliferation of mesenchymal cells	Chondrogenesis	Cartilage maturation	Cartilage resorption Bone formation	Bone remodeling

This sequence of biological events triggered by BMP at an ectopic site recapitulates the different stages of endochondral ossification, as observed during embryonic skeletal development or fracture repair [20].

Sampling technique

The parameters measured in our experimental protocol (hematobiochemical examination) included: leukocytes, hemoglobin, blood platelets, calcium, phosphorus, and alkaline phosphatase. These parameters were assessed for both groups: the control group and the group where the allografts were coated with EEPLA (ethanolic extract of Laghouat Algerian propolis). After each blood sample collection, the tubes were sent to a private hematobiochemical analysis laboratory located in the Tiaret province. The method used for the complete blood count (CBC) was the automatic counting of white blood cells and platelets, as well as hemoglobin measurement by an automated system (Mithyc 22, Switzerland). The biochemical analyses (calcium, phosphorus, and alkaline phosphatase) were performed based on colorimetric principles using an automated system (Targa 1500, Italy).

The right saphenous vein region of each animal was properly shaved and disinfected with alcohol. A layer of 2% Xylocaine gel was then applied to the area to prevent the sensation of the needle prick and minimize trauma to the blood vessels (FIGS. 2A and 2B).

Blood collection of 0.5–5mL from rabbits can be performed safely. A 2.5mL syringe was used for blood sampling, with 2mL of blood being collected at each operation. This was placed into heparinized tubes for biochemical analysis and Ethylenediaminetetraacetic acid (EDTA) tubes for complete blood count (CBC) [21].

Statistical analysis

To compare the variations in hematobiochemical parameters for the control group (AWP) and the propolis–treated group (ACWP), we applied a statistical method deemed appropriate for this purpose: the comparison of means using analysis of variance (ANOVA). For calculation and analysis, we used version R-5.2 of the R software (R Development Core Team, 2005). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

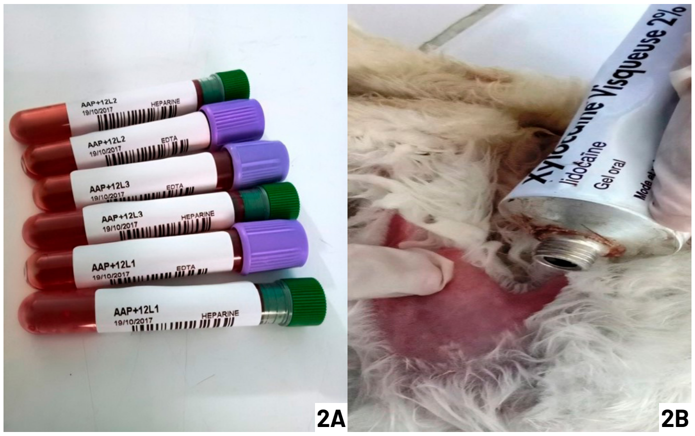

FIGURE 2. A: Labeled blood collection tubes. B: Shaving and application of 2% Xylocaine gel

RESULTS AND DISCUSSION

The present study aimed to target a few hematobiochemical parameters (hemoglobin, leukocytes, platelets, alkaline phosphatase, calcium, and phosphorus) to monitor the osseointegration of autoclaved allogeneic grafts, both covered and uncovered with EEPLA.

Comparison of AWP/ACWP

Hemoglobin

In FIG. 3, it shows non–significant variations in hemoglobin levels for the AWP and ACWP groups after the surgical procedure. The results from the analysis of hematobiochemical parameters revealed several key insights regarding the effects of propolis on post–operative recovery and healing.

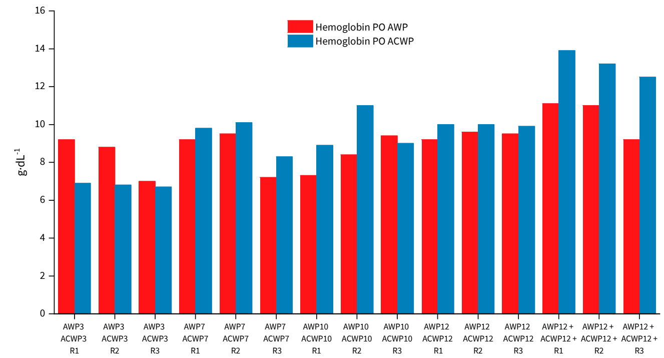

FIGURE 3.Variation in Hemoglobin levels in post–op. *P<0.05; **P<0.01; ***P<0.001

Hemoglobin levels in both the AWP and ACWP groups showed significant variations in both pre – and post–operative phases, with a decrease in hemoglobin levels due to blood loss during surgery. This decrease aligns with previous studies [22], but post–operative recovery was observed in the +12 groups, suggesting improved healing over time. However, a comparison of hemoglobin levels between the two groups in the post–operative phase yielded non–significant results, likely due to similar surgical procedures and comparable blood loss in both groups.

Leucocytes

In FIG. 4, shows a highly significant variation (P<0.001) in leukocyte levels observed after the surgical procedure for both the ACWP and AWP groups, with normal values (2,000-15,000·mm^{-3}) for the ACWP group.

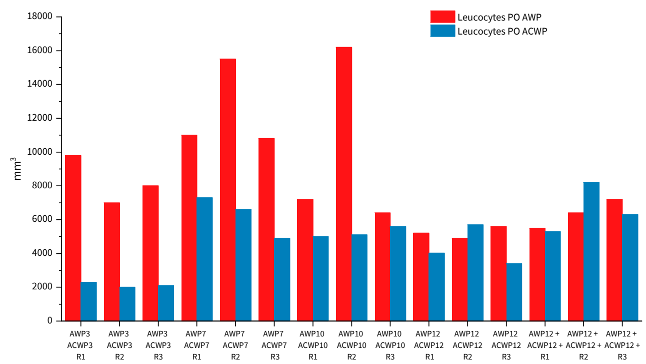

FIGURE 4. Variation in Leucocyte levels in post–op. *P<0.05; **P<0.01; ***P<0.001

Leukocyte counts were notably higher in the AWP group post–operatively, which may indicate an inflammatory or infectious response to the surgical trauma, particularly the osteotomy performed. In contrast, the ACWP group exhibited a significant reduction in leukocyte levels (P<0.01), supporting the idea that propolis has an immunostimulatory effect, as evidenced by previous research [23].This decrease could also be linked to the local immune–modulating properties of propolis, which may have helped regulate the inflammatory response. Additionally, the recruitment of blood leukocytes to the graft, perceived as a foreign body by the immune system, may have contributed to these observations.

Platelets

The variations in platelet levels for the ACWP and AWP groups were highly significant (P<0.001) after the surgical procedure, even exceeding the normal values (120,000-800,000·mm^{-3}) for the ACWP group (case ACWP12R2) (FIG. 5).

Platelet counts showed significant variation in the ACWP group (P<0.001), whereas the AWP group showed no significant changes. The presence of propolis likely contributed to this difference, as it contains anti–platelet aggregation compounds, particularly flavonoids and polyphenols, which are known to inhibit platelet activation and aggregation [24]. This anti–aggregatory effect may

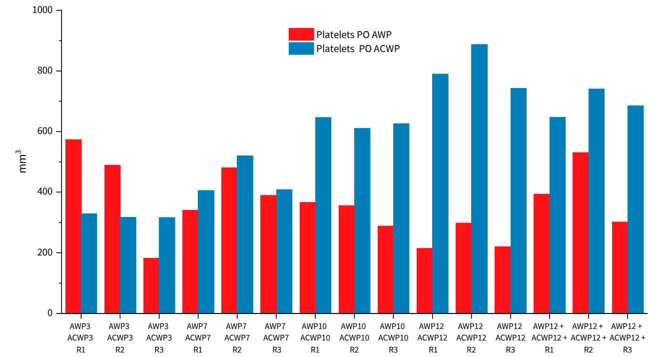

FIGURE 5. Variation in Platelet levels in post–op. *P<0.05; **P<0.01; *** P<0.001

have helped modulate platelet activity during the post–operative phase, potentially reducing the risk of excessive clot formation or microthrombi. Excessive platelet aggregation may impair local microcirculation, thereby reducing oxygen delivery to the graft site. This is particularly relevant since oxygen tension plays a pivotal role in mesenchymal stem cell differentiation: elevated oxygen levels promote osteoblastogenesis, whereas hypoxic conditions favor chondroblast formation [16]. By mitigating unnecessary platelet aggregation and preserving microvascular perfusion, propolis may indirectly enhance oxygenation at the graft site, thus supporting osteogenic differentiation and contributing to bone graft consolidation. Furthermore, given that platelets are key players in multiple thrombotic disorders, their controlled modulation is increasingly being considered in therapeutic strategies [25] , further reinforcing the rationale for using natural anti–aggregatory agents such as propolis in surgical settings.

Alkaline phosphatase

The comparison of serum alkaline phosphatase enzymatic activity for the group of animals that received a graft covered with propolis and the group without propolis in the post–operative phase revealed results with highly significant variations (P<0.001) (FIG. 6).

The activity of alkaline phosphatase, an enzyme produced by osteoblasts and associated with bone formation, was significantly elevated in both pre – and post–operative phases for the ACWP group (P<0.001). This suggests that propolis may

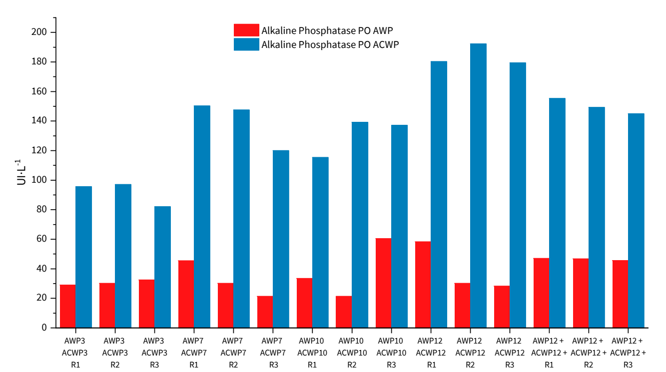

FIGURE 6. Variation in Serum Alkaline Phosphatase levels in post–op. *P<0.05; **P<0.01; *** P<0.001

have an osteoinductive effect, promoting osteoblast activity and facilitating bone healing, a finding that aligns with the role of alkaline phosphatase in bone regeneration [2].

Calcium

FIGURE 7 indicates highly significant variations (*P*<0.001) in calcium levels in the post–operative phase for both groups with and without propolis, with an increase in calcium levels in the ACWP group compared to the AWP group.

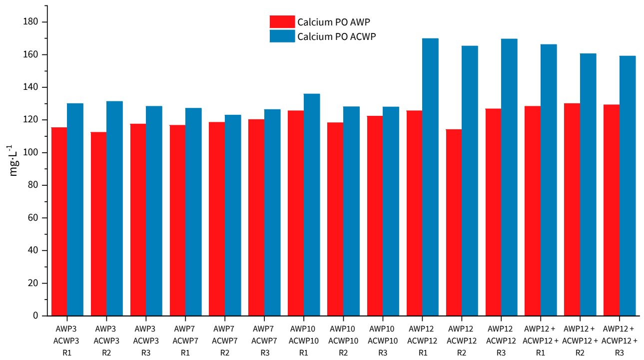

FIGURE 7. Variation in Calcium levels in post–op. *P<0.05; **P<0.01; *** P<0.001

Calcium levels showed no significant changes in either group, which can be attributed to the unique characteristics of calcium metabolism in rabbits. Unlike many other species, calcium absorption in rabbits is not dependent on vitamin [26]. According to the study by Lany *et al.* [27], although calcium and vitamin D supplementation had a marked effect on the mineral properties of bones in osteoporotic rabbits, the bone response was not strictly correlated with vitamin D levels. Vibrational spectroscopy analyses revealed that changes in bone mineralization could occur even with variable vitamin D intake, suggesting the presence of a specific regulatory mechanism for calcium in rabbits, potentially less reliant on vitamin D compared to other species. Consequently, blood calcium concentrations remained relatively stable despite the surgical intervention, highlighting a fundamental difference in calcium homeostasis in rabbits, with minimal impact from surgical procedures.

Phosphorus

FIGURE 8 displays results with non–significant variations in phosphorus levels in both groups (EEPA 10-covered graft and non–propolis–covered graft) in the post–operative phase.

Phosphorus levels were significantly lower in the AWP group post–operatively, which may be due to the cortical bone osteotomy, potentially disrupting the bone's mineral content. However, for the ACWP group, phosphorus levels remained stable, suggesting that propolis may have contributed to replenishing phosphorus levels. The mineral content of propolis, as noted by Nedji and Loucif–Ayad [28], could have played a role in maintaining the phosphorus balance in the ACWP group, possibly helping to mitigate any deficiencies caused by the osteotomy.

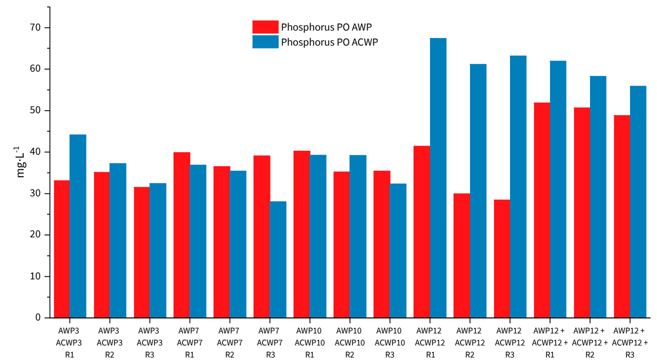

FIGURE 8. Variation in Phosphorus levels in post–op. *P<0.05; **P<0.01; *** P<0.001

CONCLUSION

Hematobiochemical results show that EEPLA (Laghouat) has an immunostimulatory effect, aiding in post–surgical recovery. While blood loss during surgery led to decreased hemoglobin levels, propolis appears to enhance recovery. It also contains anti–platelet aggregation compounds, potentially reducing the risk of excessive clotting. Additionally, it appears that calcium metabolism in rabbits is independent of vitamin D, which is different from other species and may impact future research on calcium regulation in rabbits. The significant increase in alkaline phosphatase activity in the ACWP group suggests that propolis promotes osteoinduction and bone healing. Moreover, the stable phosphorus levels in the ACWP group indicate that propolis may help maintain phosphorus balance, supporting bone repair.

ACKNOWLEDGMENTS

The authors wish to express their sincere gratitude to the Laboratory of Hygiene and Animal Pathology, Institute of Veterinary Sciences, University of Tiaret, for their invaluable support and assistance throughout the course of this study.

Conflict of interest

The authors declare no conflicts of interest related to this report

Funding

No funding was received for this study.

BIBLIOGRAPHIC REFERENCES

[1] Jain J, Kumar S, Aggarwal AN, Jajodia N. Augmentation of bone healing in delayed and atrophic nonunion of fractures of long bones by partially decalcified bone allograft (decalcified bone). Indian J. Orthop. [Internet]. 2015; 49(6):637-642. doi: https://doi.org/gjp37z

[2] Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. J. Orthop. Surg. Res. [Internet]. 2014; 9(1):18. doi: https://doi.org/gbftx2

[3] Wang W, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: A review. Bioact. Mater. [Internet]. 2017; 2(4):224-247. doi: https://doi.org/gdg7mk

[4] Kresnoadi U, Widjaja J, Laksono H. Ethanol extract of propolis–bovine bone graft combination as a prospective candidate for socket preservation: Increase of BMP7 and decrease of NFATc1. Saudi Dent. J. [Internet]. 2021; 33(8):1055-1062. doi: https://doi.org/pvt5

[5] Boudra A, Hamdi M, Amara K. Role of propolis in osteointegration enhancement in allograft autoclaved implanted in femur of dog. J. Vet. Sci. Technol. [Internet]. 2014; 5:173. doi: https://doi.org/pvt6

[6] Sowmya S, Gujjari AK, Mruthunjaya K, Sushma R, Ravi MB, Swamy RKN. Effect of propolis on bone loss prevention – A review. J. Evol. Med. Dent. Sci. [Internet]. 2017; 6(93):6785-6790. doi: https://doi.org/pvt7

[7] Boudra A, Merati R, Meliani S, Aggad H. The effect of Algerian propolis in the incorporation of diaphyseal autoclaved allografts in rabbits. FEB [Internet]. 2023 [cited Feb. 22, 2025]; 32(10):3157-3166. Available in: https://goo.su/JzfwZ

[8] Meimandi–Pariz A, Oryan A, Sayahi AO, Bigham–Sadegh A. Propolis extract a new reinforcement material in improving bone healing: An *in vivo* study. Int. J. Surg. [Internet]. 2018; 56:94-101. doi: https://doi.org/pvt8

[9] Berroukche AA, Terras M, Slimani BM, Denai I. Characterization of bioactive compounds in South–Western Algeria propolis samples. J. New Technol. Mater. [Internet]. 2017; 7(2):67-71. doi: https://doi.org/pvt9

[10] Aliakbarian M, Shahraki Jazinaki M, Bahari H, Rashidmayvan M, Golafrouz H, Khodashahi R, Pahlavani N. Effect of propolis consumption on liver enzymes and obesity indices in adults: A systematic review and dose–response meta–analysis. Curr. Dev. Nutr. [Internet]. 2024; 8(9):104438. doi: https://doi.org/pvvb

[11] Lahouel M, Boutabet K, Kebsa W, Alyane M. Polyphenolic fractions of Algerian propolis reverse doxorubicin induced acute renal oxidative stress. Afr. J. Pharm. Pharmacol. [Internet]. 2010 [cited Feb. 21, 2025]; 4(10):712-720. Available in: https://goo.su/6yqU64R

[12] Hendi NKK, Naher HS, Al–Charrakh AH. In vitro antibacterial and antifungal activity of Iraqi propolis. J. Med. Plants Res. [Internet]. 2011[cited Feb. 21, 2025]; 5(20):5058-5066. Available in: https://goo.su/lKvh

[13] Pereira YCL, Issa JPM, Watanabe E, Nascimento GC, Iyomasa MM, Ciampo JOD, Ervolino E. The therapeutic use of propolis extract in alveolar bone contaminated with bacterial endotoxin. Dent. [Internet]. 2018; 8(3): 1000473. doi: https://doi.org/pqwx

[14] Boudra A, Benbelkacem I, Aissa MA. Antibacterial activity of different ethanolic extracts of Algerian propolis against *Staphylococcus aureus*. Bionature [Internet]. 2020 [cited Feb. 23, 2025]; 40(1):4-8. Available in: https://goo.su/FwxtChz

[15] Lipman N, Marini RP, Erdman SE. A comparison of ketamine/xylazine and ketamine/xylazine/acepromazine anesthesia in the rabbit. Lab. Anim. Sci. [Internet]. 1990 [cited Feb. 22, 2025]; 40(4):395-398. PMID: 2166867. Available in: https://goo.su/kPMByo

[16] Brinker WO, Piermattei DL, Flo GL. Manual of Orthopedics and Fracture Treatment in Small Animals. 2nd ed. Paris: Éditions du Point Vétérinaire; 1994. p. 71-77.

[17] Kılıç N. A comparison between medetomidine–ketamine and xylazine–ketamine anaesthesia in rabbits. Turk. J. Vet. Anim. Sci. [Internet]. 2004 [cited Jan. 15, 2025]; 28(5):921-926. Available in: https://goo.su/MOJEB

[18] Asmara AGY, Kamal AF, Siregar NC, Prasetyo M. Effect of autoclave devitalization on autograft incorporation and bone morphogenetic protein of tibia in Sprague–Dawley rats. Med. J. Indones. [Internet]. 2014; 23(2):74-78. doi: https://doi.org/pvwr

[19] Boudra A, Benbelkacem I, Merati R, Achour H, Daouadji ID. Comparison between three fixed anaesthesia protocols in rabbits. J. Prev. Vet. Med. [Internet]. 2020; 44(3):99-103. doi: https://doi.org/pqw3

[20] Sautier JMF, Forest N. Bone morphogenesis proteins: BMP. Med. Sci. (Paris). 1996; 12:364-370.

[21] Boucher S, Nouaille L. Rabbit Diseases. 3rd ed. France: France Agricole Editions; 2013. 356 p.

[22] Liumbruno GM, Bennardello F, Lattanzio A, Piccoli P, Rossetti G. Recommendations for the transfusion management of patients in the peri–operative period. III. The post–operative period. Blood Transfus. [Internet]. 2011; 9(3):320-335. doi: https://doi.org/pvws

[23] Ezgi A, Hasan Y. Evaluation of the effect of propolis on implant stability by resonance frequency analysis and removal torque test. Biomed. J. Sci. Tech. Res. [Internet]. 2017; 1(5):1-6. doi: https://doi.org/pvwt

[24] Zhang YX, Yang TT, Xia L, Zhang WF, Wang JF, Wu YP. Inhibitory effect of propolis on platelet aggregation *in vitro*. J. Healthc Eng. [Internet]. 2017; 2017:3050895. doi: https://doi.org/gcjszr

[25] Tian Y, Zong Y, Pang Y, Zheng Z, Ma Y, Zhang C, Gao J. Platelets and diseases: signal transduction and advances in targeted therapy. Signal Transduct. Target. Ther. [Internet]. 2025; 10:159. https://doi.org/pvwv

[26] Jekl V, Redrobe S. Rabbit dental disease and calcium metabolism – the science behind divided opinions. J. Small Anim. Pract. [Internet]. 2013; 54(9):481-490. doi:https://doi.org/pvww

[27] Lani A, Kourkoumelis N, Baliouskas G, Tzaphlidou M. The effect of calcium and vitamin D supplementation on osteoporotic rabbit bones studied by vibrational spectroscopy [Internet]. J. Biol. Phys. [Internet]. 2014; 40:401–412. doi: https://doi.org/f6n6tk

[28] Nedji N, Loucif–Ayad W. Antimicrobial activity of Algerian propolis in foodborne pathogens and its quantitative chemical composition. Asian Pac. J. Trop. Dis. [Internet]. 2014;4(6):433-437. doi: https://doi.org/pvwx