

Original article

Histomorphometric Evaluation of Wormwood Effects on Cellular Activity: An *In-Vitro* Experimental Study

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Antioxidant, Inflammation.**ABSTRACT**

Artemisia absinthium L. (Asteraceae), commonly known as wormwood in the United Kingdom and absinthe in France, is an aromatic, perennial small shrub. It is locally known as 'chajret mariem' in Tunisia. The herb has always been of great botanical and pharmaceutical interest and is employed in folk medicine against various pains [11]. Wormwood essential oil has been widely used mainly for its neuroprotective, antifungal, antimicrobial, and antidepressant properties. This study aimed this study to explain the beneficial effect of wormwood on inflammation in the oral tissue after extraction. A total of 24 adult male rats aged about 6 months and weighing about 250 g were divided into 2 groups. Group I (12 rats) non diabetic. Group II (12 rats). The study group was exposed to surgical wounds (extracted lower right first molar). They were sacrificed as follows: 4 rats from each group at intervals of 3 days, 7 days, and 21 days after extraction for histomorphometric study. In the present study, significant changes were observed in the mean number of active cells during the study period between 3 days and 7 days ($p_1 = 0.043$), and no significant changes between 3 days and 21 days ($p_1 = 0.109$). Whereas, no significant changes were observed in the mean number of active cells during the study period between 7 days and 21 days $p_2 (= 0.588)$. The current results demonstrate that Wormwood decreased the inflammatory signs, leading to shortening of the healing time, which means that the wormwood has a well-known antiseptic property.

Introduction

A complex pathophysiological process including several cellular and biochemical sub-processes. e.g., inflammation, angiogenesis, and collagen deposition. Inflammation and inadequate vessel formation comprise the most noticeable causes of delayed wound healing [1]. Plant extracts have been widely used as a topical application for wound healing and disease treatments. These plants share a common character; they all produce flavonoid compounds with phenolic structures. These phytochemicals are highly reactive with other compounds, such as reactive oxygen species and biological macromolecules, to neutralize free radicals or initiate biological effects [2].

Artemisia absinthium L. (Asteraceae), commonly known as wormwood in the United Kingdom and absinthe in France, is an aromatic, perennial small shrub. It is locally known as 'chajret mariem' in Tunisia. The herb has always been of great botanical and pharmaceutical interest and is employed in folk medicine against various pains [3]. Wormwood essential oil has been widely used mainly for its neuroprotective [4], antifungal [5], antimicrobial [6], and antidepressant [7] properties. The therapeutic potential of Artemisia absinthium L (wormwood) is attributed to a complex mixture of bioactive constituents, notably polyphenolic acids, flavonoids, and essential oil components [8]. Preclinical evidence indicates that the chemical composition of Artemisia absinthium can modulate central wound-healing pathways. For instance, in vitro studies demonstrate that wormwood extracts promote keratinocyte proliferation and migration [9], while in vivo models show they reduce oxidative stress-1 and accelerate wound contraction [10]. These observed effects on specific cell types and physiological processes support the hypothesis that systemic administration of a standardized extract may favorably influence the cellular microenvironment of a healing extraction socket. Aromatic and medicinal plants are an easily accessible and edible source of natural antioxidants. They can protect the organism from damage caused by free radical-induced oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases [11,12]. While no research specifically on tooth extraction sockets was found, studies on other types of wounds show that Artemisia absinthium (wormwood) can actively promote wound healing by directly stimulating skin cells and creating a healthy healing environment. This is achieved through a combination of cellular, antibacterial, and antioxidant effects. The objective of this study is to explain the beneficial effect of wormwood on the activity of fibroblast and osteoblast cells.

Methods

Twenty-four adult male rats weighing about 200-250 gms, and aged about 6 months, were used in this study. These animals were obtained from the Institute of Medical Research, Tripoli University. Animals were housed in specially designed wire mesh bottom cages, three animals per cage. All the animals were supplied with a regular and the same diet ad libitum throughout the whole experimental period. The study included the following groups: Group I: (12 rats) was given 50µL/Kg distilled water by gastric tube/day. Group II: (12 rats) were given 50µL/Kg of 5% wormwood extract by gastric tube/day.

Preparation of 5% of wormwood extract (*Artemisia herba-alba*)

Wormwood was available at Aromatic shops □. Five (5)g of the wormwood leaf, steeped for 1.5-2 minutes in 100 ml of distilled water. The coolest brewing temperature will be 160F°/69 C°. The mixture was purified to obtain a 5% concentration of wormwood.

Data collection procedure

For the study group

Each animal received general anaesthetic solutions of 10% (40 ml/k.gm body weight) and zylazine 2% (5 mg/k.gm body weight). The surgical site was disinfected using an iodine swab, extraction of the mandibular right 1st molar of all animals was carried out. four animals in each group were sacrificed at 3-, 7-, and 21-days following tooth extraction. Jaws were dissected, and specimens were fixed in 10% formalin saline for 7 days to be prepared for a Histomorphometric study.

Results

Histomorphometric analysis (The number of active fibroblast and osteoblast cells)

Table 1 shows the comparison between the different studied periods according to the number of active cells. The 1st reading represents the mean number of active cells for group I during the different studied periods. Significant changes were observed in the mean number of active cells during the study period between 3 days and 7 days ($p_1 = 0.043$), and no significant changes between 3 days and 21 days ($p_1 = 0.109$). Whereas, no significant changes were observed in the mean number of active cells during the study period between 7 days and 21 days ($p_2 = 0.588$). The second reading represents the mean number of active cells for group II during the different studied periods. Significant changes were observed in the mean number of active cells during the study period between 3 days and 7 days ($p_1 = 0.043$), and no significant changes between 3 days and 21 days ($p_1 = 0.138$). whereas no significant changes were observed in the mean number of active cells during the study period between 7 days and 21 days ($p_2 = 0.893$).

Table (1). Comparison between the different studied periods according to the number of active cells

	Number of active cells (After extraction)		
	3 days (n = 4)	7 days (n = 4)	21 days (n = 4)
Group I			
Min. – Max.	1.0 – 3.0	4.0 – 11.0	1.0 – 12.0
Mean ± SD.	2.20 ± 1.10	7.0 ± 2.92	5.80 ± 4.55
Median	3.0	6.0	4.0
p ₁		0.043*	0.109
p ₂			0.588
Group II			
Min. – Max.	4.0 – 14.0	20.0 – 35.0	9.0 – 46.0
Mean ± SD.	10.20 ± 3.77	26.20 ± 6.30	20.40 ± 14.62
Median	11.0	25.0	15.0
p ₁		0.043	0.138
p ₂			0.893

p₁: p-value for the Wilcoxon signed-rank test for comparing the 3 days and each other in two groups.

p₂: p-value for the Wilcoxon signed-rank test for comparing between 7 days and 21days in two groups

*: Statistically significant at $p \leq 0.05$.

Table 2 shows the Comparison between the studied groups according to the number of active cells 3 days after extraction. The 1st reading for comparing group I and group II on 3 days after extraction, no significant changes were observed in the mean number of active cells during the study period, 3 days between group I and group II ($p = 0.169$).

Table (2). Comparison between the studied groups according to the number of active cells 3 days after extraction

Number of active cells	Group I (n = 4)	Group II (n = 4)	KW χ^2
3 days after extraction			
Min. – Max.	4.0 – 17.0	14.0 – 31.0	
Mean \pm SD.	11.60 \pm 5.03	18.20 \pm 7.19	6.945
Median	12.0	15.0	
p		0.169	

KW χ^2 : Chi-square for Kruskal-Wallis test, pairwise between groups using the Mann-Whitney test. p: p-value for comparing group I and group II. *: Statistically significant at $p \leq 0.05$.

Table 3 shows the Comparison between the studied groups according to the number of active cells 7 days after extraction. The 1st reading for comparing group I and group II on 7 days after extraction, significant changes were observed in the mean number of active cells during the study period, 7 days between group I and group II ($p = 0.009$).

Table (3). Comparison between the studied groups according to the number of active cells 7 days after extraction

Number of active cells	Group I (n = 4)	Group II (n = 4)	KW χ^2
7 days after extraction			
Min. – Max.	9.0 – 18.0	20.0 – 35.0	
Mean \pm SD.	14.20 \pm 3.42	26.20 \pm 6.30	14.241*
Median	15.0	25.0	
p		0.009*	

KW χ^2 : Chi-square for Kruskal-Wallis test, pairwise between groups using Mann-Whitney test
p: p-value for comparing group I and group II
*: Statistically significant at $p \leq 0.05$

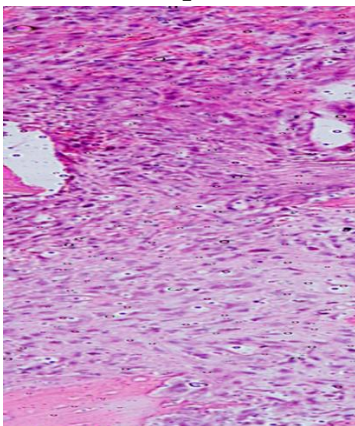
Table 4 shows the Comparison between the studied groups according to the number of active cells 21 days after extraction. Comparing group, I and group II on 21 days after extraction, significant changes were observed in the mean number of active cells during the study period, 21 days, between group I and group II ($p = 0.009$).

Table 4. Comparison between the studied groups according to the number of active cells 21 days after extraction

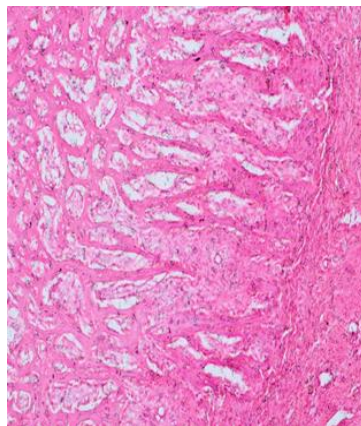
Number of active cells	Group I (n = 4)	Group II (n = 4)	KW χ^2
21 days after extraction			
Min. – Max.	7.0 – 16.0	27.0 – 46.0	
Mean \pm SD.	12.20 \pm 3.70	38.60 \pm 7.83	13.145*
Median	13.0	40.0	
p		0.009*	

KW χ^2 : Chi-square for Kruskal-Wallis test, pairwise between groups using Mann-Whitney test. P: p-value for comparing group I and group II. *: Statistically significant at $p \leq 0.05$.

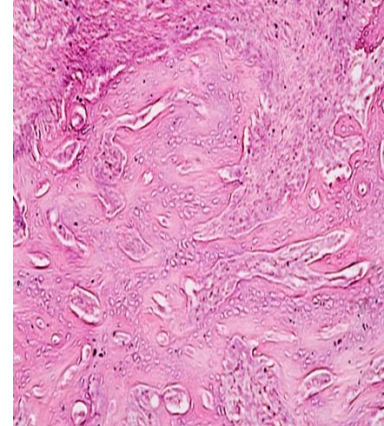
Control Group



3 Days Figure(A)



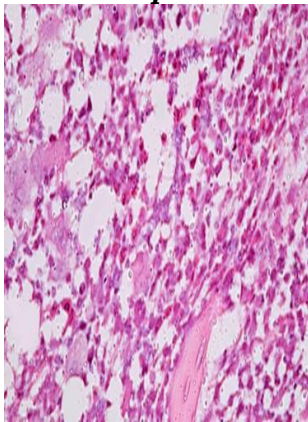
7 Days Figure (B)



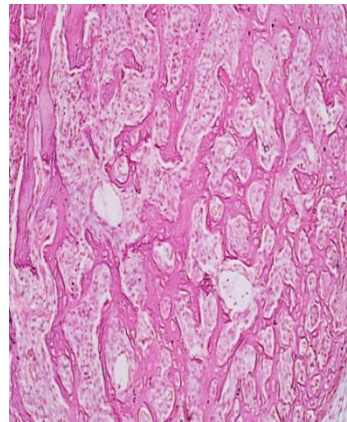
21 Days Figure(C)

- (A) A light micrograph, Extraction site of group I after 3 days showing: proliferative phase of fibroblastic activity and formation of granulation tissue with some inflammatory cells (H&E x100).
- (B) A light micrograph, extraction site of group I after 7 days, showing narrow bone trabeculae (woven bone) lined with osteoblast cells on both sides of the trabeculae. Granulation tissue and bundles of collagen fibers are inserted into the newly formed bone (H&E X100).
- (C) A light micrograph, extraction site of group I after 21 days showing thicker bone trabeculae encircling marrow spaces, osteocytes in their lacunae scattered in between bone trabeculae, and granulation tissue in the upper part of the socket (H&E × 100).

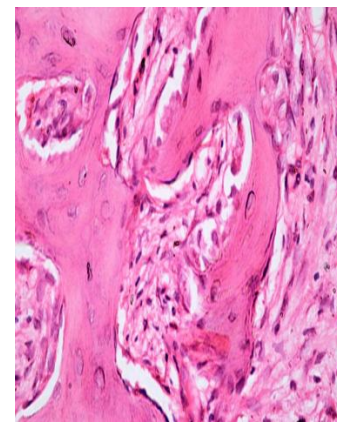
Wormwood Group



3 days Figure (A)



7 days Figure (B)



21 days Figure (C)

- (A) : High power view showing collagen fiber with fibroblastic activity, formation of extracellular matrix, and immature vascularity (H&E x400).
- (B) : A light micrograph, extraction site of group II after 7 days, showing the socket filled with thicker bone trabeculae than group I, a small area of granulation tissue with fibroblastic activity found in the superficial part of the socket (H&E x100)
- (C) : High power view showing well-organized osteoblastic activities lining the bone trabeculae and osteocytes in their lacunae. Notice the bundles of collagen fibers inserted into the surface of the bone, and incremental lines are evident (H&E x 400).

Discussion

Sequence of cellular and biochemical responses directed toward restoring tissue integrity and functional capacity following injury. Although healing occurs uneventfully in most instances, a variety of intrinsic and extrinsic factors can impede or facilitate the process [13]. Many factors can interfere with this process, thus causing improper or impaired wound healing. These factors include poor oxygenation, infection, age, stress, diabetes, obesity, medications, alcoholism, smoking, and poor nutrition [14].

Numerous studies report the antioxidant effects of Wormwood. Wormwood extract has been investigated for its antifungal [5], antimicrobial [6], and antidepressant [7]. However, little work has been done to examine the effect of wormwood extract on healing. So, the present study aimed to evaluate the effect of wormwood extract on the healing of oral tissues in rats. The structure of alveolar bone in rats is similar to a great extent

to that in human beings [15]; therefore, the former has been used in this study to examine the healing of the extraction socket.

The number of active cells during healing in this study was measured at intervals throughout the experimental period. The number of these active healing cells showed a statistically significant increase in the wormwood group (II). where the wormwood group showed an increase in the number of healing cells & improved epithelial formation when compared to control animals. These results were supported by many studies that have reported antioxidant and antibacterial properties of wormwood [11,16].

In the present study, group II (wormwood group) showed an increased number of fibroblast and osteoblast cells, where the socket in 7 days was filled with thicker bone trabeculae and vascularity compared with group I. Also, incremental lines were more numerous in group II than in group I, indicating remodeling processes and bundles of collagen fibers compared to group I. The improvement in the healing in group II compared to group I may be due to the antioxidant and anti-inflammatory effect of this plant (Wormwood) that induces changes in the number of active cells. The findings also may be due to the antioxidant effects of the wormwood compounds [11]. This may explain the enhanced number of fibroblast cells and osteoblast cells of group II in this study compared to group I.

Conclusion

From the result of the current work can be concluded that wormwood decreased the inflammatory signs, leading to shortening of the healing time, that is to means that the wormwood has a well antiseptic property. I recommend studying the effect of awormwood on periodontal disease and using it as a mouthwash. It is important to note that while the results are encouraging, these findings are largely based on preclinical(animal) studies; further clinical trials are necessary to fully validate the efficiency and safety of wormwood-based products for human wound care.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

References

1. Appleton I. Wound healing: future directions. *J Drugs Dermatol*. 2003;6:1067–72.
2. Katiyar SK, Afaq F, Perez A, Mukhtar H. Green tea polyphenol epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet B-induced pyrimidine dimers in DNA. *Clin Cancer Res*. 2001;6:3864–86.
3. Bora KS, Sharma A. The genus *Artemisia*: a comprehensive review. *Pharm Biol*. 2011;49(1):101–9.
4. Bora KS, Sharma A. Phytochemical and pharmacological potential of *Artemisia absinthium* Linn. and *Artemisia asiatica* Nakai: a review. *J Pharm Res*. 2010;3(2):325–8.
5. Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *J Agric Food Chem*. 2005;53(24):9452–8.
6. Juteau F, Jerkovic I, Masotti V, et al. Composition and antimicrobial activity of the essential oil of *Artemisia absinthium* from Croatia and France. *Planta Med*. 2003;69(2):158–61.
7. Mahmoudi M, Ebrahimzadeh MA, Ansaroudi F, Nabavi S, Nabavi SM. Antidepressant and antioxidant activities of *Artemisia absinthium* L. at flowering stage. *Afr J Biotechnol*. 2009;8(24):7170–5.
8. Batiha GES, Olatunde A, Al-Rejaie S, Saad AS, El-Mleeh A, Helal H, et al. Bioactive compounds, pharmacological actions, and pharmacokinetics of wormwood (*Artemisia absinthium*). *Antibiotics (Basel)*. 2022;9:1–25.
9. Minda D, Ghiulai R, Banciu CD, Pavel IZ, Danciu C, Racoviceanu R, et al. Phytochemical profile, antioxidant and wound healing potential of three *Artemisia* species: in vitro and in ovo evaluation. *Appl Sci*. 2022;12(3):1–20.
10. Boudjelal A, Smeriglio A, Ginestra G, Denaro M, Trombetta D. Phytochemical profile, safety assessment and wound healing activity of *Artemisia absinthium* L. *Plants*. 2020;9(12):1–14.
11. Jiratanan T, Liu RH. Antioxidant activity of processed table beets (*Beta vulgaris* var. *conditiva*) and green beans (*Phaseolus vulgaris* L.). *J Agric Food Chem*. 2004;52(9):2659–70.
12. Losso JN, Shahidi F, Bagchi D. Anti-angiogenic functional and medicinal foods. Boca Raton (FL): Taylor & Francis; 2007.
13. Guo S, DiPietro LA. Factors affecting wound healing. *J Dent Res*. 2010;89:219–29.
14. Bergman SA. Perioperative management of the diabetic patient. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;103:731–7.
15. Travagli V, Zanardi I, Valacchi G, Bocci V. Ozone and ozonated oils in skin diseases: a review. *Int J Ozone Ther*. 2010;9:109–17.
16. Wright CW. *Artemisia*. New York (NY): Taylor & Francis; 2002.