

Cannabis extracts as modulators of bone mineralization and regenerative repair: Evidence from a Zebrafish Model

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Abstract

Background: Cannabis-derived constituents can modulate vertebrate tissue regeneration, offering mechanistic insight into how bioactive compounds influence limb- or fin-like skeletal structures. Zebrafish fin regeneration is a well-established model for studying blastema dynamics, osteoblast function, and mineralization programs, and is sensitive to chemical perturbations that affect morphogenesis and mineral deposition. Cannabis components Tetrahydrocannabinol (THC), Cannabidiol (CBD), and terpenes are known to elicit distinct developmental and morphogenetic effects in zebrafish models, with evidence for entourage-like interactions and varied impacts on mineralization and tissue architecture.

Objective: To quantify regenerative length as a proportion of pre-amputation fin across a 15 days' timeline in a controlled zebrafish caudal-fin amputation model, comparing mock controls to three cannabis-associated exposure paradigms (Δ^9 -THC, CBD, terpenes). To delineate how constituent classes influence wound healing morphogenesis, mineralization, and regenerative architecture relative to controls, and to explore potential timing and composition-dependent interactions.

Methods: Using a standardized amputation protocol, adult zebrafish were allocated to mock, THC, CBD, or terpenes treatment groups within a multi-component exposure framework. Fin regeneration was quantified as percent restoration of the original fin length at serial time points (up to 15 days). Histology of crushed lepidotrichia and Alizarin Red staining assessed mineralization and ray repair. Group differences were evaluated via multi-time-point comparisons, with reported significance ($p < 0.0001$) at multiple time points.

Results: Controls followed canonical regeneration with rapid outgrowth and patterning; $>50\%$ restoration early post-amputation and near-complete regeneration later. Terpenes produced the strongest impairment (final $\approx 40\%$ of original fin), followed by CBD, then THC (terpenes $>$ CBD $>$ THC). Early points ($\leq$ day 5) showed THC-related attenuation of impairment relative to CBD, indicating distinct temporal control of regeneration by each constituent class. Histology revealed convergence of repair signals across groups, but terpene-treated fins displayed hollow-like morphologies and node-like disruptions, while THC/CBD-exposed fins exhibited intensified Alizarin Red staining in crush zones, suggesting altered mineralization despite reduced gross regeneration.

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Conclusion: Cannabis-derived constituents modulate fin morphogenesis and mineralization, with terpene-rich exposures most disruptively influencing regenerative architecture. The findings support entourage-like interactions shaping vertebrate bone/regenerative responses and underscore exposure composition and timing as critical determinants of regenerative outcomes. Future work should probe terpene-driven osteogenic signaling and blastemal patterning mechanisms across regimens. Since heterotopic ossification involves dysregulated osteogenic signaling and ectopic mineralization, understanding how cannabinoids modulate these pathways could help identify potential targets to prevent or slow pathological bone growth. Although the current findings are preliminary and derived from a regenerative model, they provide a foundation for future studies investigating whether cannabinoid-related compounds could play a role in controlling or modulating the biological processes underlying heterotopic ossification.

Keywords: Zebrafish; THC; CBD; Terpenes; Caudal Fin; Regeneration

1. Introduction

The biological effects of marijuana and its active components tetrahydrocannabinol (THC) and cannabidiol (CBD) are increasingly recognized in scientific research and clinical applications, particularly concerning their roles in tissue regeneration and bone healing. As cannabis legalization progresses for both medical and recreational use, understanding the therapeutic potential of these cannabinoids is critical for optimizing treatment strategies.

THC and CBD exert their effects through interaction with the endocannabinoid system (ECS), primarily through specific receptors such as the cannabinoid receptors CB1 and CB2. THC is well-known for its psychoactive effects, while CBD is favored for its therapeutic benefits devoid of significant psycho-activity [1]. This distinction is pivotal, particularly in the medical field, where CBD is emerging as a promising agent for various health issues related to inflammation and tissue repair without the risks associated with THC-induced psycho-activity.

A primary mechanism by which CBD promotes healing is its anti-inflammatory action. Multiple studies have illustrated that CBD can effectively reduce inflammation, which is vital for enhancing the healing environment. By modulating various inflammatory mediators, including cytokines, CBD aids in creating conditions conducive to tissue regeneration [2-3]. For instance, the suppression of inflammatory markers such as IL-6 and TNF- α has been documented, with CBD playing a critical role in this modulation [3]. By diminishing inflammatory responses, CBD may enhance recovery processes and facilitate better outcomes in tissue healing.

CBD is also seen to stimulate the activity of mesenchymal stem cells (MSCs), which are integral to regenerative medicine. Studies demonstrate that CBD can promote MSC proliferation and differentiation into osteoblasts, the cells responsible for bone formation [3-4]. This stem cell activity suggests that CBD could be utilized not only in enhancing existing healing processes but also in potentially driving new tissue formation. For example, CBD has been shown to activate p42/44 MAPK pathways in MSCs, boosting their differentiation capabilities [5]. Furthermore, Kogan et al. revealed that CBD enhances fracture healing by stimulating lysyl hydroxylase activity, which is crucial for collagen synthesis, indicating its role in improving the structural integrity of newly formed tissue [4].

In contrast, THC presents a more complex interaction with biological processes related to regeneration. Although THC provides essential analgesic effects, its chronic use may hinder tissue healing and bone regeneration. Research indicates that THC can downregulate osteoblast differentiation, potentially impairing bone healing processes. Sophocleous et al. noted that heavy THC usage correlates with lower bone mineral density and prolonged healing times, raising significant concerns about its use in clinical settings [1-2]. Thus, while THC may alleviate pain associated with injuries or surgeries, its implications for bone health complicate its therapeutic applications [1-2].

Observational studies have indicated that even moderate chronic THC use can impair healing, underscoring the need for a balanced approach to cannabinoid therapies that weigh the benefits against potential risks [1-2]. The adverse effects of THC highlight the necessity for carefully weighing the therapeutic benefits and limits of cannabinoid formulations, especially in contexts requiring optimal tissue repair.

Beyond THC and CBD, terpenes organic compounds contributing to the aroma and flavor of cannabis also hold promise for influencing healing processes. Terpenes are noted for their anti-inflammatory and antimicrobial properties, suggesting they may enhance the therapeutic effects of cannabinoids through synergistic mechanisms [6]. Preliminary investigations reveal that terpenes could modulate cell migration and proliferation, which are critical processes for regeneration [6].

In recent years, researchers have begun exploring the capacity of CBD to influence osteogenic differentiation specifically within the inflammatory microenvironment, a critical area considering the prevalence of inflammation in various injury contexts [3]. The migration and differentiation of MSCs can be significantly affected by the local environment, a relationship that CBD appears to enhance positively [3, 7]. This furthers our understanding of how cannabinoids can be effectively integrated into therapies aimed at maximizing healing and regeneration.

CBD's influence extends into other biological domains, such as oral health, where it has been shown to promote the healing of periodontal tissues and support dental stem cell health [8]. The application of CBD in dental practices points to its broader implications in tissue regeneration, expanding beyond traditional perceptions of CBD and THC therapies.

Overall, the accumulated evidence suggests that cannabinoids, particularly CBD, may possess significant abilities to foster healing and tissue regeneration through multiple mechanistic pathways. Continued exploration of these cannabinoids and their interactions—including with terpenes—will likely unveil further potential applications in regenerative medicine, thereby enhancing therapeutic strategies in various clinical contexts. Collaborative efforts among researchers, clinicians, and policymakers will be essential in harnessing the promising effects of cannabinoids in medicine.

Zebrafish (*Danio rerio*) have gained attention as powerful models for evaluating the effects of cannabinoids, notably tetrahydrocannabinol (THC) and cannabidiol (CBD), particularly in the context of tissue regeneration. Their remarkable regenerative capabilities, exemplified by the ability to regenerate fins and bones, make them ideal for studying regenerative mechanisms *in vivo*. As research continues to expand on the impacts of cannabis-derived compounds on regeneration, zebrafish serve as significant subjects.

Studies indicate that exposure to THC and CBD influences regenerative processes in zebrafish. For instance, Carty et al., 2017 demonstrated that these cannabinoids could alter locomotor behavior and gene expression in zebrafish, revealing insights into their effects on growth and nervous system functions [9]. Additionally, Licitra et al., 2022 observed that cannabinoids could modulate developmental processes in zebrafish, suggesting a biphasic or hermetic effect on physiological responses [10]. Furthermore, Carty et al., 2019 noted that THC exposure could lead to nonmonotonic effects on inflammation and gene expression, emphasizing the complex interactions between cannabinoids and physiological processes [11].

The research by Tesoriere et al., 2025 highlighted the critical role of molecular pathways in zebrafish tail fin regeneration, demonstrating that these pathways are essential for understanding regeneration, which could be influenced by cannabinoid interactions [12]. Moreover, Bohaud et al., 2022 pointed out the ability of cannabinoids to affect cell proliferation during fin regeneration, drawing interest to the involvement of macrophages in this process [13]. In summary, zebrafish represent a versatile model for exploring how cannabinoids influence tissue regeneration, paving the way for future translational applications in regenerative medicine. Therefore, primary objective of the current study is to investigate the effects of marijuana's active components on caudal fin regeneration and bone fracture healing in zebrafish.

2. Materials and Methods

2.1. Chemical and equipment's

THC (60%, 600 mg/ g extract from 125 g in 10 ml) CBD (680 mg/g extract from 125 g in 10 ml) and Terpenes extract from 125 g in 10 ml) were gift from Dr. Machel Anthony Emanuel cannabis research laboratory, faculty of Science and technology, The university of the west indies mona campus, Kingston Jamaica. API Melafix antibacterial solution, API Pimafix (antifungal solution) were purchased from local aquarium store. 2-Phenoxyethanol, Potassium hydroxide, phosphate buffer saline and Alizarin red dyes were obtained from Sigma-Aldrich. The equipment used were scalpel, blue lab combo meter (pH meter), Andonstar microscopic webcam, glass plate, hot plate, HD720 webcam, laptop running iSpy software and Fiji ImageJ software.

2.2. Zebrafish Maintenance and Selection

Adult zebrafish (*Danio rerio*), averaging 3 cm in length and weighing about 700 mg, were sourced from a local aquarium store. Prior to introducing the fish, 3 g of artificial instant ocean salts were added per liter of deionized distilled water for conditioning. This preparation was done on a large scale (200L) and allowed it to stabilize over three days. Healthy adult zebrafish were selected using specific criteria and kept in large tanks measuring 45×60×25 cm. They were housed in conditioned water with a conductivity of about 1,500 $\mu\text{S}/\text{cm}$ and a pH of 7. The temperature was maintained at 28°C,

with continuous aeration provided by an air pump to ensure proper oxygen levels. Water quality was managed through daily two-hour filtration and the addition of aquarium disinfectants. Additionally, 30% of the tank water was replaced daily with Millipore-filtered water containing the appropriate amount of instant ocean salt. The fish were fed three times daily (at 10 am, 2 pm, and 6 pm) with bloodworms, enough to be consumed within five minutes. After ten days of maintenance, zebrafish with similar morphology and length, having equal caudal fin length and exhibiting normal swimming and feeding behaviors, were chosen and housed in 3 pairs separately in 1.5 L Yobiisolut mini aquarium tanks for further study.

2.3. Anesthetization, Crush of Zebrafish caudal lepidotrichia and Caudal Fin Amputation

Adult zebrafish were anaesthetized in 0.015% 2-Phenoxyethanol in fish water. For this a 1% stock solution of 2-phenoxyethanol was prepared by mixing 5 mL of 100% pure 2-phenoxyethanol with 500 mL of distilled water. For anesthetic use, 6 mL of this stock solution was added to 400 mL of fish water. Individual fish were carefully removed from their tanks and placed in a tank containing a 0.015% 2-phenoxyethanol anesthetic solution. Anesthesia was confirmed by the absence of tail reflex. Each fish was then positioned on a petri dish, dried, and photographed with an Andonstar microscopic webcam to document the uncut caudal fin.

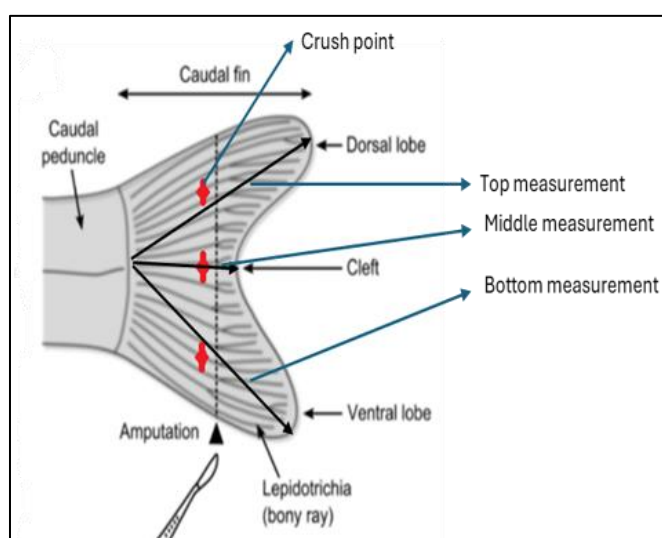


Figure 1 Sketch of caudal fin showing the position of amputation and crush points of lepidotrichia (red). Arrow (black) originated from same point indicated measurement of caudal fin

Approximately half of the caudal fin (as shown in Figure 1) was excised using a scalpel, and a photograph was taken immediately afterward in facility water and placed on a petri dish under dissecting stereomicroscope. Excess water was removed by paper tissue, the fish was positioned on its side, and the tail was spread out evenly on the surface. Drummond number 5 forceps were used to perform a bone crush of lepidotrichia. 3 crushes per tail were introduced, on medial, dorsal and ventral sides of the tail at 2mm behind amputated caudal fin, with the minimal strength needed to crush without impacting adjacent tissues. Fish were returned to a tank filled with standard fish tank water for recovery.

2.4. Tail collection and storage

Following crush fracture, tails were collected at the required repair stage by anaesthetization in buffered Tricaine and dissection of the whole tail fin with a scalpel. Collected tails were placed in 4% paraformaldehyde (PFA) solution and kept at 4°C, with shaking, overnight. The following day, specimens were washed in PBST briefly, then moved through a methanol series consisting of 30, 60 and 100% methanol. Specimens were kept for 10 min in each solution and then stored in 100% methanol at -20°C.

2.5. Alizarin Red bone staining

Specimens stored in 100% methanol at -20°C were moved through a methanol/PBS wash series consisting of 10 min washes in 100, 60 and 30% methanol, followed by two 10 min PBST washes. Subsequently, samples were pre-incubated in 0.5% KOH for 15 min and moved to 0.2% Alizarin Red solution in 0.5% KOH for overnight staining at room temperature. Next day, three sets of 5 min washes with 0.5% KOH were performed followed by 1h hydrogen peroxide

bleaching (3% H₂O₂ in 1% KOH). Upon completion, three sets of 10 min PBST washes were performed, and samples were moved to glycerol for storage and imaging.

2.6. Treatment of Zebrafish with THC, CBD and Terpene

Zebrafish caudal injuries were studied in groups of six fish each in triplicate, with crushed caudal lepidotrichia followed by caudal fin amputation. The experimental subjects were exposed to condition water containing a single-cabinet mixture of THC, CBD, and terpenes (in DMSO) at an initial concentration of 5 mg/L, administered twice daily for 5 days. The final DMSO concentration in these treatment solutions was maintained below 0.01% to avoid DMSO-associated toxicity in zebrafish [14]. Following the initial 5-day course, the same cohort received an escalated dose of 10 mg/L THC, CBD, and terpenes, again applied twice daily for 5 days. A final treatment phase employed 15 mg/L THC, CBD, and terpenes for an additional 5 days, continuing the same administration schedule. The cumulative protocol demonstrated effectiveness, with pre-exposure to the lower THC dose enhancing survival at the higher exposures (10 and 15 mg/L), compared with direct exposure to 10 or 15 mg/L THC alone, in which higher mortality occurred before the 15-day endpoint for evaluating caudal fin growth and healing of crushed lepidotrichia, consistent with prior findings [15].

2.7. Data collection and statical evaluation

Caudal fin regeneration in zebrafish were quantified using a standardized sequence of injury and measurement. Baseline fin length was established on 18 fish per group following anesthesia. The caudal fin was subjected to two injuries: crushing of the caudal lepidotrichia and proximal amputation. Fin length was remeasured at 5-day intervals to monitor regrowth, with a final assessment on day 15. At this point, a 3 mm segment proximal to the crushed region was amputated for detailed analysis. Amputated fins were fixed and stained with Alizarin Red to visualize mineralized tissue, and the morphology of lepidotrichia was captured via high-resolution imaging. Data are reported as mean $\pm$ standard deviation (SD). Statistical analyses were conducted in GraphPad Prism.

3. Results

The measurement on percentage regeneration as described in method section is shown in Figure 2. As can be seen from the figure, cannabis-derived constituents THC, CBD, and a terpene-rich fraction (Terpenes) greatly influence caudal fin healing and bone repair in zebrafish. Across the experimental groups, the treated fins exhibited a pronounced suppression of regenerative length relative to controls ($p < 0.0001$) at all time points, indicating robust impairment of fin outgrowth by cannabis-derived exposures. The mock-treated controls achieved substantial regeneration, surpassing 50% early after amputation and approaching near-complete restoration by later stages, consistent with the well-established rapid regenerative phases in zebrafish caudal fins. This baseline trajectory aligns with the canonical model wherein post-amputation regeneration proceeds through initiation, blastema formation, accelerated outgrowth, and eventual patterning over approximately two to three weeks, suggesting our 90% restoration observation remains within expected physiological bounds for unexposed individuals [16]. However, in the THC-, CBD-, and Terpenes-treated groups, regenerated fin length was markedly diminished: Terpenes produced the strongest suppression, with final regenerated lengths clustering around 40% of the original fin, whereas THC and CBD showed progressively milder reductions, yet still significantly lower than controls. At early time points (up to day 5), THC-treated fins displayed less attenuation than CBD-treated fins, while Terpenes consistently exhibited the greatest inhibition of regenerative length, indicating a rank order of effect: Terpenes > CBD > THC in terms of impairment, with THC exerting the mildest impact among the three treatments.

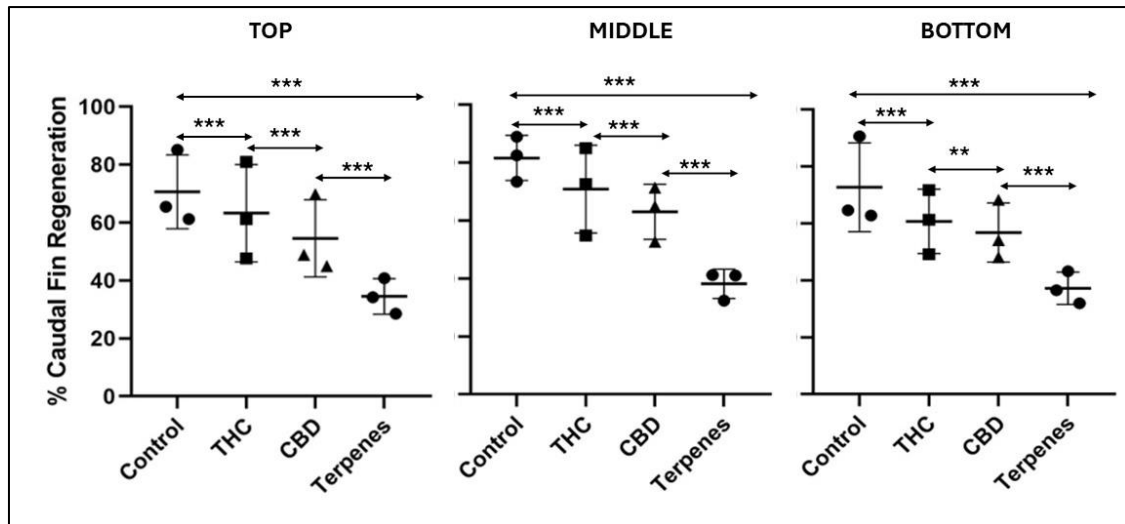


Figure 2 Regeneration of the zebrafish caudal fin under exposure to THC, CBD, and terpenes across fin regions (Top = dorsal lobe, Middle = mid-region, Bottom = ventral lobe). In the dorsal and ventral lobes, control and CBD-alone conditions show an initial stall in outgrowth between days 5 and 10 of treatment, followed by renewed growth after day 10; THC-alone treatment, in contrast showed uniform progressive outgrowth in all time points. CBD appears to transiently dampen regeneration, with later recovery observed like controls. The middle region exhibits a parallel regenerative trajectory under control, THC, and CBD treatments, suggesting region-specific responses or buffering by neighboring tissues. Terpenes, however, markedly suppress outgrowth across all regions after an initial regeneration phase, achieving about 30% regeneration before stalling persists. Each data point represents the mean of 18 measurements from three replicates of six fish each; the lowest, middle, and top data points correspond to 5, 10, and 15 days of treatment, respectively. Across treatment groups, fins exposed to cannabis-derived exposures show a pronounced reduction in regenerative length relative to controls at all time points ($p < 0.0001$), indicating robust impairment of fin outgrowth by cannabis constituents.

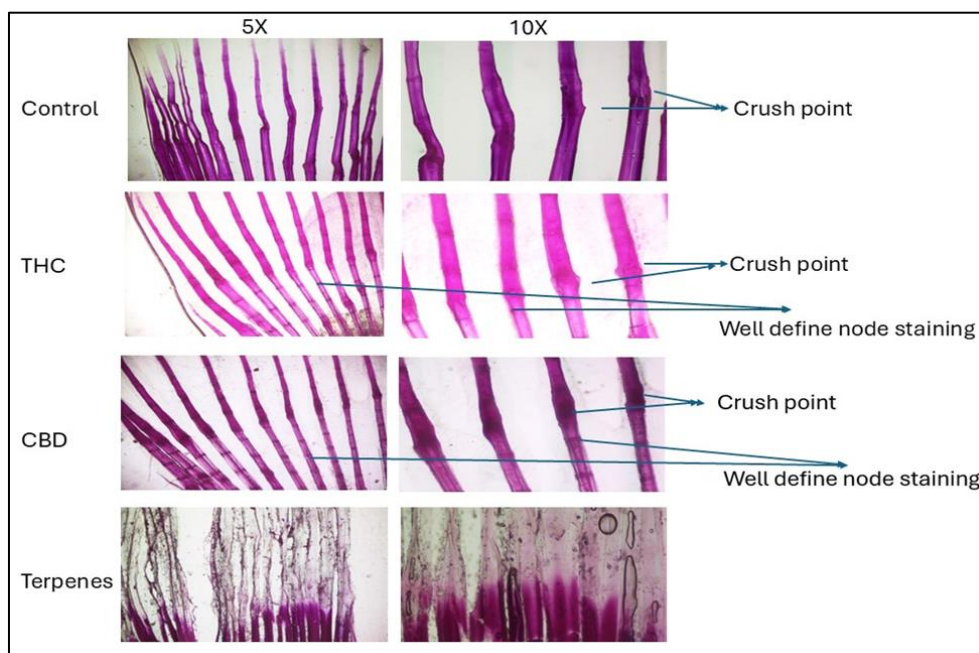


Figure 3 Alizarin Red staining of lepidotrichia of Zebrafish caudal fin and visualization of crushed lepidotrichia heading and outgrowth of amputated lepidotrichia after 15 days treatment with THC, CBD and Terpenes

Histological assessment of crushed lepidotrichia revealed convergent indicators of repair attempts across groups (Figure 3), including fixation of crushed elements and thinner diameters with smoother outgrowth patterns, but with hollow-like morphologies and node-like disruptions more prevalent in terpene-treated fins. In THC- and CBD-exposed specimens, Alizarin Red staining intensified within the crush zone, accompanied by increased lepidotrichia diameter and smoother regrowth, suggesting enhanced mineralization activity at these injury sites despite overall diminished regeneration. In contrast, THC- and CBD-treated fins retained more organized mineralization signatures than Terpenes-treated fins, where large-diameter outgrowths were observed but with weak Alizarin Red uptake and less distinct patterning in the regenerated tissue mass.

Beyond gross morphology, the observations imply differential engagement of inflammatory and osteogenic pathways. THC- and CBD-exposed fins showed heavy mineralization in repair zones, consistent with modulated osteogenic activity, while terpene exposure appeared to antagonize orderly skeletal patterning and produce less pronounced mineral labeling.

Collectively, these histological patterns corroborate the functional data: cannabis constituents can modulate mineralization dynamics and morphogenesis of lepidotrichia during repair, with terpene-rich fractions driving the most disrupted regenerative architecture [17-19].

4. Discussion

The investigation into the effects of cannabinoid and terpene compounds on caudal fin healing in zebrafish sheds light on the complexity of phytochemical interactions and their physiological implications. Cannabidiol (CBD), tetrahydrocannabinol (THC), and terpenes exhibit varied effects on regenerative processes, influencing both soft and hard tissue dynamics. Examination reveals that these compounds uniquely engage with regenerative mechanisms during fin healing, demonstrating significant alterations that produce distinct outcomes.

4.1. THC and CBD are the paradoxical enhancers of bone structure but inhibitors of healing progression:

Tetrahydrocannabinol (THC) presents an intriguing paradox in its effects on fin healing. Evidence supports that THC facilitates certain structural aspects of bone healing, notably increasing callus density and mineral content while concurrently impeding overall healing progression [20-21]. Specifically, THC exposure is found to result in heightened fin thickness and increased mineral staining, indicative of enhanced collagen matrix formation and greater mineral recruitment [21-22]. This behavior may be attributed to THC's activation of osteogenic signaling pathways, potentially functioning via the CB2 receptor, which mediates osteoblast differentiation and subsequent bone formation [23-24]. Such activation correlates with findings that THC influences collagen cross-linking, effectively generating a more rigid and compact callus [21, 25].

However, this structural advancement comes at the cost of healing efficiency. THC's known anti-inflammatory properties may play a crucial role, as early inflammatory responses are integral for successful blastema formation and tissue regeneration during the early healing stages [20]. The suppression of these inflammatory signals could lead to premature stabilization of tissue before an adequate proliferative mass has formed, resulting in a shorter yet denser repaired fin that lacks necessary flexibility for proper healing [25-26]. Hence, while THC promotes certain late-stage structural characteristics, it disrupts initial cellular events essential for effective regeneration [27-29].

CBD exhibits a more pronounced inhibition of fin healing compared to THC, while similarly increasing callus density and collagen deposition [22,26]. CBD's effects are notably stronger in promoting matrix maturation, suggesting broader interaction with various molecular pathways beyond direct CB1 and CB2 receptor activation. Studies show that CBD modulates the balance between osteoclastogenesis and osteogenesis, favoring bone formation [22]. Despite these enhancements in matrix characteristics, CBD's effect also constitutes a premature acceleration into late-stage healing, hindering the required flexibility for successful regeneration [22, 27-29].

The cumulative effects of both THC and CBD highlight the critical nature of timing and coordination in the regenerative processes. Although these compounds bolster structural components, they violate the developmental timetable crucial for effective healing, leading to adverse outcomes such as stunted tissue growth and compromised functional restoration.

4.2. Terpenes, the distinct suppressors of healing and bone quality

Conversely, the exposure of zebrafish fins to terpene compounds yields a distinctive set of results, characterized by impaired healing and diminished mineralization [26]. The structural features of the fin, including callus thickness and

collagen density, are notably reduced in the presence of terpenes, which not only slow down healing but also compromise essential bone formation processes [30]. Their interaction with regenerative mechanisms is understood partly through their anti-inflammatory and antioxidative properties, yet their precise impact on osteogenic pathways remains less elucidated [30].

The reduced outgrowth and solely superficial healing of fins treated with terpenes suggest that these compounds could potentially interfere with cytokine signaling or other crucial regulatory factors involved in matrix synthesis and osteoblast recruitment [22, 30]. This suppressive effect stands in stark contrast to the behaviors elicited by THC and CBD, which enhance certain pathways while simultaneously obstructing healing. The terpene-induced dampening of both early proliferative and late-stage mineralization processes points to a fundamentally different biological profile, underscoring the nuanced interplay of cannabis phytochemistry with skeletal repair mechanisms [27-29,31-32].

4.3. Comparative interplay of THC, CBD, and Terpenes in fin healing

The interactions among THC, CBD, and terpenes in the context of caudal fin healing illustrate a gradient of effects on tissue regeneration. THC is associated with moderate healing suppression while still promoting increased callus density and mineralization, albeit through mechanisms that may disrupt normal inflammatory responses and collagen cross-linking processes [20,26]. CBD, while enhancing matrix maturation, exerts the strongest inhibitory effects on healing, leading to early transitions into stiffness that limit regenerative potential [22-26]. In comparison, terpenes uniquely suppress both healing and bone mineralization, affecting essential processes that drive early proliferation and late-stage mineralization [26].

Thus, the findings elucidate that cannabinoids and terpenes do not exert homogenous or synergistic effects during the healing process. Instead, each class of compounds targets different stages of tissue regeneration, with THC and CBD pushing the timeline of healing forward at the cost of proper tissue formation, while terpenes indiscriminately slow down the entire healing process [31,33].

4.4. Implications for bone biology and cannabinoid research

The implications of these findings extend beyond mere observations into pivotal understandings of skeletal biology, regenerative medicine, and cannabinoid research. First, the observation that structural enhancements do not equate to improved healing is critical. Increased thickness and mineral content, as facilitated by cannabinoids, do not necessarily correlate with functional healing progress nor imply that an adequate biological framework for regeneration has been established [20,26,27-29]. Second, the detrimental role of premature ossification, where mineralization occurs ahead of patterning and proliferation, exemplifies a significant barrier to effective regenerative outcomes [22].

Furthermore, the distinctive roles of non-cannabinoid cannabis components, like terpenes, reveal an additional layer of complexity within the cannabis plant's biological profile. Their capacity to dampen mineral formation presents significant avenues for future research, especially given the broad use of terpene-rich cannabis products [30]. This evidence accentuates the necessity for a contextual understanding of the endocannabinoid system's engagement with skeletal healing processes, as variables such as timing, receptor distribution, and the specific concentrations of cannabinoids and terpenes significantly influence whether bone formation yields positive or negative outcomes [20, 22].

5. Conclusion

In conclusion, the study provides important insights into how THC, CBD, and terpenes uniquely influence the biological interplay underlying caudal fin healing in zebrafish. Each compound exhibits distinct characteristics that modulate the healing process in multifactorial and highly context-dependent manners. THC and CBD serve primarily to perturb healing advancements despite enhancing specific structural facets, while terpenes present a unique profile of suppression across both healing and mineralization pathways. Collectively, this body of work emphasizes the importance of detailed mechanistic exploration before considering the therapeutic implications of cannabis-derived compounds in contexts of bone repair and regeneration. By demonstrating that THC, CBD, and terpenes can alter bone mineralization and skeletal healing processes, the results suggest that cannabis-derived compounds might influence abnormal bone formation in soft tissues. Since heterotopic ossification involves dysregulated osteogenic signaling and ectopic mineralization, understanding how cannabinoids modulate these pathways could help identify potential targets to prevent or slow pathological bone growth. Although the current findings are preliminary and derived from a regenerative model, they provide a foundation for future studies investigating whether cannabinoid-related compounds could play a role in controlling or modulating the biological processes underlying heterotopic ossification.

Compliance with ethical standards

Disclosure of conflict of interest

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Statement of ethical approval

Ethical approval was granted by the Mona Campus Research Ethics Committee (Ref: CREC- AN.001, 2023/2024).

Authors' Contribution

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- Nyron Nembhard: Research Assistant and data collection.
- Shahane Stephenson: Research Assistant and data collection.
- Wayne St. Orville Palmer: Research coordinator
- Machel Anthony Emanuel: Cannabis constituent extraction and quantification
- Mohammad Kutub Ali: Research supervisor and coordinator, data analyzer and manuscript drafting.

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