



EFFECT OF GENTAMICIN - *NIGELLA SATIVA* FUSION
EMULSIONS ON OSTEOLAST CELL LINE FOR USE
IN OSTEO-HEALING APPLICATIONS

BY

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ABSTRACT

An alternative osteo-healing formulation with osteo-healing properties was formulated by combining gentamicin and *Nigella sativa* oil (NSO) in a form of gentamicin-*N. sativa* fusion emulsion (GNFE). This work aims to formulate a stable emulsion and to study the effects of GNFE on UMR-106 osteoblast-like rat osteosarcoma cell line *in vitro* and its related mechanisms of bone healing and regeneration. Emulsion A, B, C and D had been formulated, with final concentration of gentamicin was made constant at 0.1%, whereas NSO concentration was varied at 32.5%, 35.0%, 40.2% and 46.4% in all formulations respectively. Stability studies of emulsion A, B, C and D were performed at different storage conditions (8°C, 25°C and 50°C), followed by *in vitro* study of MTT assay, Alizarin Red S (ARS) staining, von Kossa staining and quantification, alkaline phosphatase (ALP) quantification and quantitation of collagen type-1 and osteocalcin (qPCR). Results showed that all emulsions were stable at storage temperature of 8°C. *In vitro* results showed that emulsion D produced the highest cell viability (97.1%) at 72 hours of post-incubation. The highest mineral deposits (2.64 ± 0.05) and ALP activity (2.19 ± 0.3 nmol) was produced by emulsion D at day 21. Lastly, the highest expression of collagen type-1 (29.4 ± 1.01 folds) and osteocalcin (1.8 ± 0.51 folds) were expressed by the cells treated with emulsion C. Thus, stable GNFE may have the ability to promote bone formation.

خلاصة البحث

تم تصنيع صيغة بديلة لعلاج العظام ذات خصائص شافية للعظم من خلال مشاركة الجنتاميسين مع زيت الحبة السوداء بشكل مستحلب اندماجي. يهدف هذا البحث إلى تصنيع مستحلب اندماجي ثابت من الجنتاميسين وزيت الحبة السوداء ودراسة تأثيراته على خلايا سرطان العظم UMR-106 في الزجاج وكذلك آليات شفاء العظم وتجديده. صنعت المستحلبات 1، ب، ج ود، بتركيز نهائي ثابت من الجنتاميسين قدره 0.1%، بينما تركيز زيت الحبة السوداء كان 32.5%، 35.0%، 40.2% و46.4% على الترتيب. أجريت دراسة الثباتية على المستحلبات ا، ب، ج ود في ظروف تخزين مختلفة (8، 25 و50 درجة مئوية)، وأتبعته بدراسة السمية الخلوية في الزجاج، صباغ الأليزارين الأحمر س، صباغ الفونكوسا مع التحديد الكمي، التحديد الكمي للفوسفاتاز القلوية و التحديد الكمي للكولاجين من النوع-1 والأوستيوكالسين (ال بي سي ر الكمي). أظهرت النتائج أن كل المستحلبات كانت ثابتة في درجة الحرارة 8 مئوية. النتائج في الزجاج أظهرت أن المستحلب د أدى إلى أعلى نسبة من حيوية الخلايا (97.1%) بعد 72 ساعة من الحضانة. أعلى نسبة من الترسب المعدني (2.64 ± 0.05) وفعالية الفوسفاتاز القلوية (2.19 ± 0.3 نانومول) تم الحصول عليها من المستحلب د في اليوم 21. في النهاية، أعلى نسبة تعبير مورثي للكولاجين نوع-1 (29.4 ± 1.01 مرة) والأوستيوكالسين (1.8 ± 0.51 مرة) تمت مشاهدتها في الخلايا المعالجة بالمستحلب ج. وبهذا يمكن أن نستنتج أن المستحلب الاندماجي الثابت من الجنتاميسين وزيت الحبة السوداء يمكن أن يكون له القدرة على تحريض تشكل العظام.

ABSTRAK

Suatu formulasi alternatif untuk rawatan ortopedik dengan ciri-ciri penyembuhan tulang telah dihasilkan dengan menggabungkan gentamicin dan minyak dari *Nigella sativa* (NSO) dalam bentuk emulsi gentamicin-*N. sativa* (GNFE). Kajian ini bertujuan untuk menghasilkan emulsi yang stabil dan untuk mengkaji kesan GNFE pada sel osteoblast UMR-106 secara *in vitro* serta mekanisme berkaitan penyembuhan tulang. Emulsi A, B, C dan D telah dihasilkan dengan kepekatan akhir gentamicin ialah 0.1%, manakala kepekatan NSO telah dimanipulasi pada 32.5%, 35.0%, 40.2% dan 46.4% dalam setiap rumusan. Kajian kestabilan emulsi A, B, C dan D telah dijalankan pada keadaan penyimpanan yang berbeza (8°C, 25°C dan 50°C), diikuti dengan kajian *in vitro* iaitu *MTT assay*, pewarnaan *Alizarin Red S* (ARS), pewarnaan dan kuantifikasi *von Kossa*, kuantifikasi *alkaline phosphatase* (ALP), dan kuantifikasi gen *collagen type-1* dan *osteocalcin* (qPCR). Hasil kajian menunjukkan bahawa semua emulsi adalah stabil pada suhu penyimpanan 8°C. Keputusan *in vitro* menunjukkan bahawa emulsi D menghasilkan daya maju sel tertinggi (97.1%) pada 72 jam selepas inkubasi. Penghasilan tertinggi deposit mineral (2.64 ± 0.05) dan aktiviti ALP (2.19 ± 0.31 nmol) telah dihasilkan oleh emulsi D, pada hari ke-21. Akhir sekali, penghasilan tertinggi *collagen type-1* (29.4 ± 1.01 folds) dan *osteocalcin* (1.8 ± 0.51 folds) telah dihasilkan oleh sel-sel yang dirawat dengan emulsi C. Oleh itu, GNFE yang stabil telah dihasilkan dan berkemungkinan mempunyai keupayaan untuk menggalakkan pembentukan tulang.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Health Sciences (Biomedical Science).

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DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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To my beloved family, friends and teachers,

To the inspired,

To everyone who's ever tried,

To those who hope,

To each of you,

Thank you.

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