

**THE EFFECT OF DATE PALM (*Phoenix dactylifera*) AND  
GOAT MILK ON IRON METABOLISM IN IRON  
DEFICIENCY ANAEMIA (IDA) INDUCED RAT**

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This thesis is submitted in fulfilment of the requirement for the degree of  
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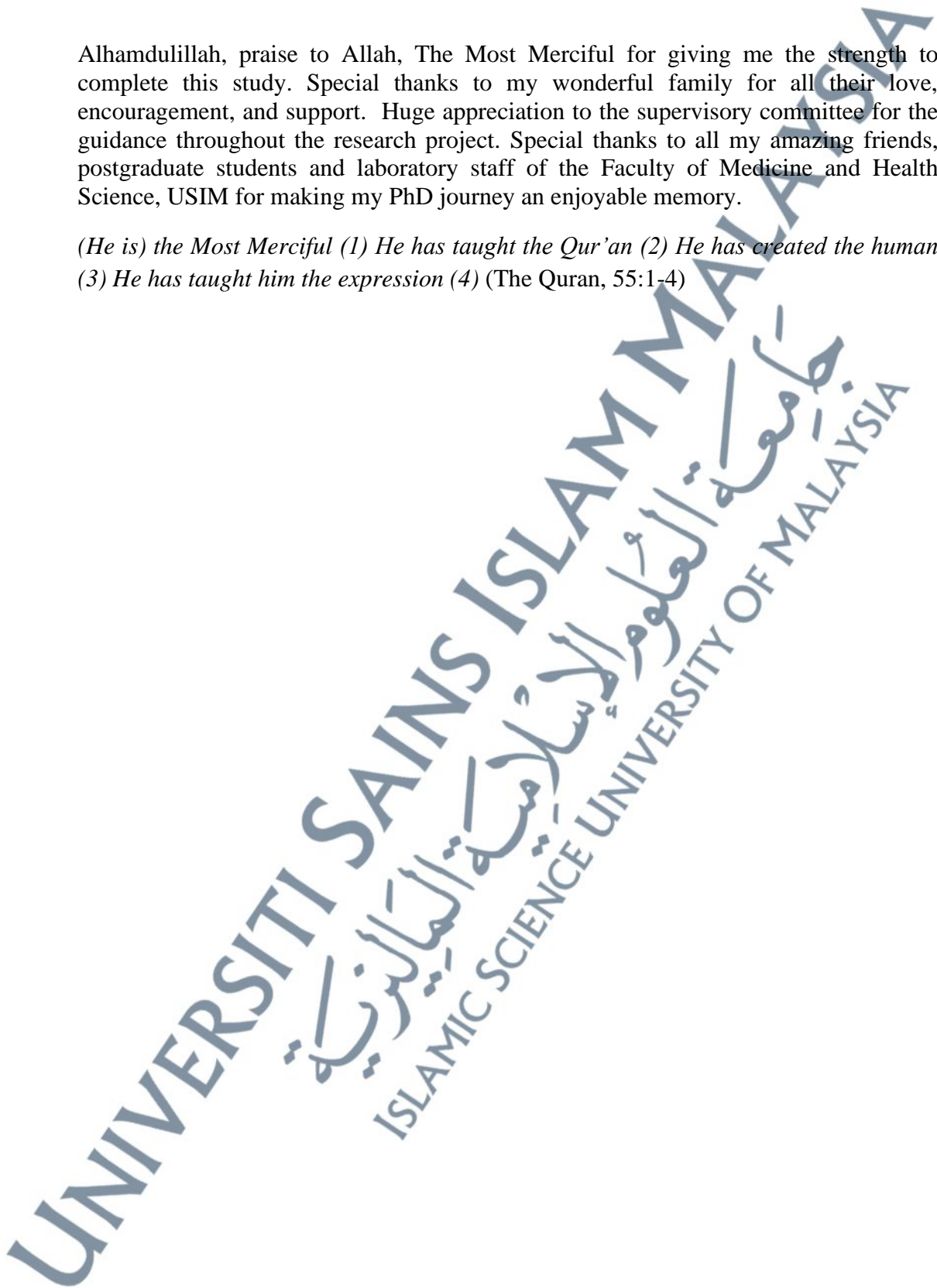
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*(He is) the Most Merciful (1) He has taught the Qur'an (2) He has created the human (3) He has taught him the expression (4) (The Quran, 55:1-4)*



## ABSTRAK

### **Kesan buah kurma (*Phoenix dactylifera*) dan susu kambing terhadap metabolisme zat besi pada tikus teraruh anemia kekurangan zat besi (IDA)**

Anemia kekurangan zat besi (IDA) adalah keadaan di mana darah tidak mempunyai sel darah merah sihat yang cukup kerana kekurangan zat besi. Pengurusan semasa IDA sering dikaitkan dengan kesan sampingan buruk dan isu kepatuhan. Tujuan kajian ini adalah untuk menerokai potensi penggunaan kurma dan susu kambing sebagai alternatif semula jadi untuk IDA. Kajian sebelum ini menunjukkan kurma dan susu kambing mempunyai sifat anti-anemia. Dalam kajian ini, tikus telah dibahagikan secara rawak kepada enam kumpulan yang mengandungi empat tikus; (1) kawalan biasa; (2) kawalan negatif; (3) kawalan positif; (4) kurma; (5) susu kambing; (6) kurma dan susu kambing. Kawalan biasa diberikan pelet komersial manakala kumpulan lain (Kumpulan 2 -6) diberi diet rendah zat besi selama dua minggu bagi menjana IDA. IDA ditentukan sebagai Hb kurang 11.5 g/dL. Selepas konformasi IDA, kawalan negatif (Kumpulan 2) diberi diet rendah zat besi dan bertindak sebagai kumpulan model IDA manakala kawalan positif (Kumpulan 3) diberi tablet besi sebagai rawatan standard IDA. Kumpulan kurma (Kumpulan 4) dan susu kambing (Kumpulan 5) masing-masing diberi kurma dan susu kambing manakala kumpulan kurma dan susu kambing (Kumpulan 6) diberi kurma dan susu kambing. Suplemen diberikan setiap pagi selama 4 minggu mengikut sukatan setara haiwan (AED). Berat badan, parameter RBC, profil besi, bioavailabiliti besi serta ekspresi gen dan protin berkaitan metabolisme besi disiasat. Selepas intervensi, terdapat peningkatan berat badan dalam semua kumpulan intervensi, dengan kenaikan 110g. Terdapat peningkatan indeks eritrosit kekurangan zat besi, dimana kumpulan intervensi merekodkan tahap Hb dan serum zat besi dari 13.85 hingga 16.18g/dL dan 29.35 hingga 31.95 $\mu$ mol/L lebih tinggi berbanding kawalan negatif ( $6.68 \pm 3.09$  g/dL;  $3.13 \pm 0.74$   $\mu$ mol/L). Peningkatan signifikan juga dilihat pada parameter RBC, PCV dan serum transferrin. Penyiataan bioavailabiliti besi menggunakan haemoglobin regeneration efficiency (HRE) menunjukkan peningkatan ketara dalam kawalan negatif dengan nilai 21.7%, berbanding kawalan biasa dengan nilai 3.7%, manakala kumpulan intervensi merekodkan nilai HRE antara 4.58% hingga 5.58%. Keputusan daripada ekspresi gen menunjukkan bahawa kekurangan zat besi meningkatkan ekspresi Dcytb, transferrin, ferroportin dan TfR mRNA sebanyak 13, 9, 1.5 dan 1.2 kali ganda, berbanding kawalan biasa. Suplemen kurma dan susu kambing selama 4 minggu berjaya menormalkan ekspresi gen-gen tersebut dalam usus kecil. Bagi organ hati, peningkatan ketara dalam ekspresi mRNA TfR sehingga 11.6 kali ganda dilihat dalam kawalan negatif berbanding kawalan biasa dan pengambilan kurma dan susu kambing menormalkan ekspresi TfR. Keputusan imunohistokimia menunjukkan ekspresi protin DMT1 dan Dcytb disepanjang *brush border membrane* enterosit epitelium selari dengan fungsinya dalam pengimportan besi. TfR juga terdapat di crypts dan villi sel epitelium, dengan konsentrasi semakin berkurangan apabila sel epitelium berhijrah secara apikal ke hujung vilus selaras dengan fungsinya dalam membekalkan besi bagi pertumbuhan sel baru. Kekurangan zat besi meningkatkan konsentrasi TfR dalam usus kecil dengan kereaktifan positif terlihat pada bahagian apikal dan basolateral vili epitelium dengan *brush border membrane* menunjukkan keputusan positif. Pengambilan kurma dan susu kambing menormalkan ekspresi TfR. Keputusan menunjukkan suplemen kurma dan susu kambing meningkatkan bioavailabiliti besi untuk meransang peningkatan erythropoiesis selepas IDA.

## ABSTRACT

### **The effect of date palm (*Phoenix dactylifera*) and goat milk on iron metabolism in iron deficiency anaemia (IDA) induced rat**

Iron deficiency anaemia (IDA) is a condition in which blood lacks adequate healthy red blood cells due to insufficient iron. The current management of IDA is often linked to adverse side effects and issues with compliance. Therefore, this study aims to explore the potential of using date palm and goat milk as natural alternative remedies to manage IDA. It has been demonstrated that date palm and goat milk exhibit anti-anaemic properties. In this study, rats were assigned randomly into six groups of four rats; (1) normal control; (2) negative control; (3) positive control; (4) date palm; (5) goat milk; (6) date palm and goat milk. The normal control was given commercial pellets whereas the rest of the groups (Groups 2 -6) were given low-iron diets for two weeks to generate IDA condition. IDA was defined as Hb values less than 11.5 g/dL. After IDA conformation, the negative control (Group 2) was continuously given a low iron diet and acted as the IDA model group while the positive control (Group 3) was given iron tablets as the standard treatment for IDA. The date palm (Group 4) and goat milk (Group 5) were given date palm and goat milk, respectively while the date palm and goat milk (Group 6) were supplemented with both date palm and goat milk. Supplementation was given every morning for 4 weeks in accordance with the animal equivalent dosage (AED). Bodyweight, RBC parameters, iron profile, iron bioavailability and iron metabolism-related genes and protein expression were investigated accordingly. After the intervention, improvement in body weight was observed in all intervention groups, with an increment of at least 110g. Up-regulation of indices of iron deficiency was noted, with intervention groups recording Hb and serum iron levels ranging from 13.85 to 16.18 g/dL and 29.35 to 31.95  $\mu\text{mol/L}$ , respectively as compared to the negative control ( $6.68 \pm 3.09$  g/dL;  $3.13 \pm 0.74$   $\mu\text{mol/L}$ ). A significant increase was also seen in RBC, PCV and serum transferrin saturation levels post-intervention. Investigation of iron bioavailability using haemoglobin regeneration efficiency (HRE) demonstrated a significant increase in negative control with a value of 21.7%, as compared to normal control with 3.7%, while intervention groups recorded HRE values ranging from 4.58% to 5.58%. Results from the gene expressions study showed that iron deficiency increased the expression of Dcytb, transferrin, ferroportin and TfR mRNA by 13, 9, 1.5 and 1.2-fold respectively, as compared to normal control. Supplementation with date palm and goat milk for 4 weeks successfully normalises the expression of those genes in the small intestine. In the liver, significant up-regulation of TfR mRNA up to 11.6-folds was seen in the negative control as compared to normal control and that the intervention of date palm and goat milk normalize the TfR expression. Immunohistochemistry results showed that DMT1 and Dcytb were expressed along the brush border membrane of epithelial enterocytes consistent with their function in iron import. TfR was expressed in the epithelial cell of the crypts and villi, with decreased intensity as epithelial cells migrated apically toward the villus tip consistent with its function in supplying iron for new cell growth. Iron deficiency significantly increases the reactivity of TfR in the small intestine with positive reactivity observed on both apical and basolateral sides of the epithelium villi with brush border membrane also stained positive. Intervention with date palm and goat milk normalises the TfR expression. The results obtained suggest that supplementation of date palm and goat milk improves functional iron availability for erythropoiesis following IDA.

## الملخص

### تأثير نخيل التمر (*Phoenix dactylifera*) وحليب الماعز على التمثيل الغذائي للحديد في فقر الدم الناجم عن نقص الحديد (IDA) الجرذ المستحث

فقر الدم الناجم عن نقص الحديد (IDA) هو حالة يفتقر فيها الدم إلى خلايا الدم الحمراء الصحية الكافية بسبب عدم كفاية الحديد. غالبًا ما ترتبط الإدارة الحالية للمؤسسة الدولية للتنمية بالآثار الجانبية السلبية وقضايا الامتثال. لذلك، تهدف هذه الدراسة إلى استكشاف إمكانية استخدام نخيل التمر وحليب الماعز كعلاجات بديلة طبيعية لإدارة المؤسسة الدولية للتنمية. وقد ثبت أن حليب النخيل والماعز يظهر خصائص مضادة لفقر الدم. في هذه الدراسة، تم تعيين الفئران بشكل عشوائي في ست مجموعات من أربعة فئران. (1) التحكم العادي؛ (2) التحكم السلبي؛ (3) التحكم الإيجابي؛ (4) كف التمر؛ (5) حليب الماعز؛ (6) حليب النخيل والماعز. تم إعطاء التحكم العادي الكريات التجارية في حين تم إعطاء بقية المجموعات (المجموعات 2-6) أنظمة غذائية منخفضة الحديد لمدة أسبوعين لتوليد حالة المؤسسة الدولية للتنمية. تم تعريف المؤسسة الدولية للتنمية على أنها قيم Hb أقل من 11.5 جم / ديسيلتر. بعد تشكيل المؤسسة الدولية للتنمية، تم إعطاء التحكم السلبي (المجموعة 2) باستمرار نظامًا غذائيًا منخفضًا من الحديد وعملت كمجموعة طراز IDA بينما تم إعطاء التحكم الإيجابي (المجموعة 3) أفراس الحديد كعلاج قياسي لـ IDA. نخيل التمر (المجموعة 4) وحليب الماعز (المجموعة 5) أعطيت نخيل التمر وحليب الماعز، على التوالي بينما تم استكمال حليب النخيل والماعز (المجموعة 6) بكل من نخيل التمر وحليب الماعز. تم إعطاء المكملات كل صباح لمدة 4 أسابيع وفقًا للجرعة المكافئة للحيوانات (AED). تم التحقيق في وزن الجسم ومعلومات RBC وملف تعريف الحديد والتوافر الحيوي للحديد والجينات المتعلقة باستقلاب الحديد وتعبير البروتين وفقًا لذلك. بعد التدخل، لوحظ تحسن في وزن الجسم في جميع مجموعات التدخل، بزيادة لا تقل عن 110 جم. ولوحظ تنظيم مؤشرات نقص الحديد، حيث سجلت مجموعات التدخل مستويات Hb والحديد في المصل تتراوح من 13.85 إلى 16.18 جم / ديسيلتر و 29.35 إلى 31.95 ميكرومول / لتر، على التوالي بالمقارنة مع التحكم السلبي ( $3.09 \pm 6.68$  جم / ديسيلتر؛  $0.74 \pm 3.13$  ميكرومول / لتر). كما لوحظت زيادة كبيرة في مستويات تشبع RBC و PCV و serum transferrin بعد التدخل. أظهر التحقيق في التوافر الحيوي للحديد باستخدام كفاءة تجديد الهيموجلوبين (HRE) زيادة كبيرة في التحكم السلبي بقيمة 21.7%، مقارنة بالتحكم العادي بنسبة 3.7%، بينما سجلت مجموعات التدخل قيم HRE تتراوح من 4.58% إلى 5.58%. أظهرت نتائج دراسة التعبير الجينية أن نقص الحديد زاد من التعبير عن Dcytb و transferrin و ferroportin و TfR mRNA بمقدار 13 و 9 و 1.5 و 1.2 مرة على التوالي، بالمقارنة مع التحكم العادي. المكملات مع حليب النخيل والماعز لمدة 4 أسابيع تطبيع بنجاح التعبير عن تلك الجينات في الأمعاء الدقيقة. في الكبد، شوهد تنظيم كبير لـ TfR mRNA يصل إلى 11.6 أضعاف في التحكم السلبي مقارنة بالتحكم العادي وأن تدخل نخيل التمر وحليب الماعز يطبيع تعبير TfR. أظهرت نتائج الكيمياء المناعية أنه تم التعبير عن DMT1 و Dcytb على طول غشاء حدود الفرشاة للخلايا المعوية الظهارية بما يتفق مع وظيفتها في استيراد الحديد. تم التعبير عن TfR في الخلايا الظهارية للخبايا والفيللات، مع انخفاض الشدة حيث تنتقل الخلايا الظهارية بشكل قمي نحو طرف الزغابة بما يتفق مع وظيفتها في توفير الحديد لنمو الخلايا الجديدة. يزيد نقص الحديد بشكل كبير من تفاعل TfR في الأمعاء الدقيقة مع تفاعل إيجابي لوحظ على كل من الجانبين القمي والحيوي من الزغابات الظهارية مع غشاء حدود الفرشاة الملون أيضًا إيجابيًا. التدخل في حليب النخيل والماعز يطبيع تعبير TfR. تشير النتائج التي تم الحصول عليها إلى أن مكملات نخيل التمر وحليب الماعز تعمل على تحسين توافر الحديد الوظيفي للإريثروبويتن بعد المؤسسة الدولية للتنمية.

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## LIST OF ABBREVIATIONS

bp	base pair
DNA	Deoxyribonucleic acid
DNase	deoxyribonuclease
Fe <sup>2+</sup>	Ferrous iron
Fe <sup>3+</sup>	Ferric iron
g	gram
Hb	Haemoglobin
HRE	Haemoglobin regeneration efficiency
IDA	Iron deficiency anaemia
ml	millilitre
mRNA	messenger RNA
ng	nanogram
PBS	Phosphate buffered saline
rpm	rotation per minute
T <sub>m</sub>	melting temperature
TfR	Transferrin receptor
μl	microliter
°C	degree Celsius

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