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Modulation of Duodenal TfR Expression in IDA Induced Rat with Date Palm and Goat Milk Intake

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ABSTRACT

Iron deficiency anemia (IDA) is a medical condition characterized by insufficient iron levels in the body, resulting in a reduced ability to produce hemoglobin (Hb), a critical component of red blood cells. IDA is often associated with chronic fatigue, impaired cognitive function, and diminished well-being. Transferrin (Tf) is one of the major proteins in iron homeostasis, responsible for transporting iron through the blood to various tissues, while its carrier protein, transferrin receptor (TfR), mediates the cellular uptake of transferrin-bound iron into the cell. This study aims to evaluate the expression of TfR mRNA and protein in the small intestine following intervention with date palm and goat milk in IDA-induced rats. Twenty-four male Wistar rats were induced with IDA for 2 weeks using a low-iron diet. Following IDA detection, rats were supplemented with date palm and goat milk, singly and in combination. After four weeks, the rats were sacrificed, and the expression of TfR mRNA and protein in the small intestine was assessed using qPCR and immunohistochemistry, respectively. Data were analyzed using SPSS 24.0, with a significance level set at $p<0.05$. Results demonstrated that date palm and goat milk significantly improved Hb, serum iron, Tf saturation levels, and modulated the expression of TfR mRNA in the IDA-induced rats. Expression of TfR on the crypt region and brush border membrane of the small intestine was normalized following intervention. The findings indicate that supplementation of date palm and goat milk improved Hb and Tf saturation levels and significantly modulated duodenal TfR expression in IDA-induced rats.

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1. INTRODUCTION

Anemia is a significant health concern both in Malaysia and on a global scale, with the National Health and Morbidity Survey (NHMS) estimating that 4.6 million people in Malaysia, constituting 21.3% of the population, are affected by this condition [1]. Globally, the World Health Organization (WHO) reported in 2019 that anemia affects 39.8% of children under the age of 5 and 29.9% of women in the reproductive age group[2]. Iron deficiency is widely recognized as the primary underlying cause of anemia [3]. This deficiency occurs when the body's iron stores become insufficient to support the production of Hb, the oxygen-carrying protein in red blood cells. As a result, the red blood cells are unable to adequately transport oxygen throughout the body, leading to the characteristic symptoms of anemia, such as fatigue, weakness, and paleness[4]. The regulation of iron in the body primarily revolves around dietary intake, absorption in the intestines, and the recycling of iron. Currently, there is no well-defined mechanism for actively removing excess iron. This strict regulation is crucial because an excess of iron can lead to the generation of harmful reactive oxygen species (ROS) [5]. The balance of iron in the body is primarily controlled at the absorption stage. Dietary iron is absorbed into the enterocytes of the small intestine through a transporter known as divalent metal transporter 1 (DMT1). Once inside the cells, iron can be used for cellular processes, stored in ferritin, or released into the bloodstream. Iron that circulates in the bloodstream binds to Tf. This Tf-iron complex then attaches to the TfR on the surface of various tissues after which the complex is endocytosed [6]. TfR, a transmembrane glycoprotein regulates the uptake of iron into the cell through receptor-mediated endocytosis of Tf-bound iron [7]. TfR is not only vital for iron uptake but also important in intestinal homeostasis. Inactivation of the TfR gene in intestinal epithelial cells results in severe disruption of the epithelial barrier and early death, indicating that TfR is also

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required for adult intestinal epithelial homeostasis [8]. The uptake of Tf-bound iron through TfR is the main source of iron for most cells as Tf is abundantly available and mostly unsaturated. Thus, it can accommodate a relatively large amount of iron in the circulation[9].

It is widely acknowledged that iron-deficiency anemia (IDA) has a substantial and adverse impact on an individual's quality of life (QoL). Recent studies have provided compelling evidence that addressing and treating IDA can lead to an improvement in QoL, irrespective of the root cause of anemia [4] – [6]. Elemental oral iron has been recommended as the first line defense against IDA and has been used as a standard prenatal care and as a prophylactic measure to alleviate anaemia in pregnant women and children [10]. However, despite iron supplementation, the poor compliance remains the main obstacle in improving the prevalence of IDA [11]–[13]. Others strategies implemented to reduce the incidence of IDA include food biofortification with micronutrient, dietary management, and intravenous iron. It is known that consuming a well-balanced diet that includes foods that are good sources of iron may help prevent IDA. Foods that are rich in iron includes red meat, liver, chicken, cockles, anchovy, fish, eggs, raisins, and green leafy vegetables [14], [15]. It's important to ensure an adequate intake of vitamin C as it enhances iron absorption from the diet [16]. It has been reported that date palm and goat milk have shown promise in addressing anemia. Previous studies have reported that date palm improves hemopoietic activity in anemic subjects, favouring recovery from IDA [17]–[20]. The positive effect of goat milk on iron absorption and bioavailability has also been studied in anemic subjects, particularly in animals[21]–[23]. The potential of these natural dietary sources suggests promising, accessible, and cost-effective solutions for addressing iron deficiency among affected individuals. These dietary interventions have the potential to influence dietary behaviours, especially in regions where date palm and goat milk are prevalent dietary staples, offering a potential remedy for widespread iron deficiency issues. Furthermore, positive outcomes might prompt consideration for integrating these dietary sources into public health initiatives or guidelines, potentially impacting policies related to nutritional programs.

Taking into account all of these reports, the present study was carried out to specifically evaluate the effect of date palm and goat milk supplementation on TfR mRNA and protein expression in the small intestine, as well as the iron status in IDA-induced rats.

2. MATERIALS AND METHODS

2.1 Experimental procedure

The handling and use of laboratory animals received the approval from Animal Research Ethical Committee of University Sains Islam Malaysia, Kuala Lumpur (USIM/AEC/AUP/2016). Twenty-four (24) male albino Wistar rats, 4 weeks old, weighing approximately 200-250g each, were obtained from the Animal House at Universiti Kebangsaan Malaysia, Kuala Lumpur. The rats were housed under ambient conditions with a temperature of 25°C and humidity ranging from 60% to 70%, following a 12-hour light/12-hour dark cycle. They had ad libitum access to rat pellets and distilled water. After a 7-day acclimatization period, the rats were randomly assigned to two groups: untreated and treated. The treated group was further divided into five subgroups, each containing four rats: (1) negative control, (2) positive control, (3) date palm, (4) goat milk, and (5) date palm and goat milk group. The untreated group served as the normal control and was provided with rat pellets, while the treated groups (Groups 1-5) were fed low-iron diets for two weeks to induce iron-deficiency anemia (IDA), defined as having Hb values of less than 11.5 g/dL. Blood was drawn for hematological studies, and Hb concentration was measured using the Diaspect Hemoglobin TM Analyzer (EKF Diagnostic, UK). After IDA detection, the negative control (Group 1) continued to receive a low-iron diet, while the positive control group (Group 2) was given iron tablets. The date palm group (Group 3) and goat milk group (Group 4) received date palm and goat milk, respectively, while the date palm and goat milk group (Group 5) was fed both date palm and goat milk supplements. These supplements were administered once every morning for 28 days, following animal equivalent dosage (AED) guidelines (Nair & Jacob, 2016). After 28 days, the rats were euthanized by cervical dislocation. Blood samples were analyzed for Hb, serum iron, and serum Tf saturation levels. The small intestines were preserved in RNAlater RNA Stabilization Reagent (Qiagen, Germany) for real-time PCR (qPCR) and in 10% neutral formalin solution for immunohistochemistry (IHC) analysis.

2.2 RNA extraction

RNA extraction was performed using the RNAeasy Mini Kit (Qiagen, Germany). Thirty milligrams (30 mg) of rat small intestine were homogenized in RLT buffer for 45 seconds at 450 rpm using the BeadBug™ Microtube Homogenizer (Benchmark Scientific, USA). The crude homogenate was then transferred into a new microcentrifuge tube and centrifuged for 3 minutes at 9447 x *g* (ThermoFisher Scientific, USA). The supernatant (lysate) was transferred into a new 1.5 mL microcentrifuge tube, and one volume of 50% ethanol was added. The solution was immediately mixed by pipetting up and down before being transferred into the RNeasy spin column placed in a 2 mL collection tube and centrifuged for 15 seconds at 9447 x *g*. The resulting flow-through in the 2 mL collection tube was discarded. Seven hundred microliters (700 µL) of RW1 buffer were added into the spin column and centrifuged for 15 seconds at 9447 x *g*. Five hundred microliters (500 µL) of RPE buffer were added into the spin column and centrifuged for 15 seconds at 9447 x *g*. Another 500 µL of RPE buffer was added to the spin column and centrifuged for 2 minutes at 5590 x *g*. The resulting flow-through in the 2 mL collection tube was discarded. The spin column was then placed in a new 2 mL collection tube and centrifuged at full speed for 1 minute. The resulting flow-through in the 2 mL collection tube was discarded. The spin column was placed into a new 1.5 mL collection tube, and 60 µL of RNase-free water was added into the spin column. It was then centrifuged for 1 minute at 5590 x *g* to elute the RNA. The final flow-through was collected, and the RNA concentration was determined before proceeding with cDNA synthesis.

2.3 Complementary DNA synthesis

cDNA synthesis was performed using the RT2 First Strand Kit (Qiagen, Germany). A separate 10 µL genomic DNA elimination mix consisting of 1 µL RNA, 2 µL Buffer GE, and 7 µL RNase-free water was mixed and incubated for 5 minutes at 42°C. Then, a 10 µL reverse transcription mix, consisting of 4 µL 5x Buffer BC3, 1 µL Control P2, 2 µL RE3 Reverse Transcriptase Mix, and 3 µL RNase-free water, was added to

the genomic DNA elimination mix. The solution was incubated at 42°C for 15 minutes before being incubated at 95°C for 5 minutes. Ninety-two microliters (92 µL) of RNase-free water were then added to the solution. The reaction was placed on ice and proceeded with real-time PCR (qPCR).

2.4 Real-time polymerase chain reaction (qPCR)

The expression of TfR mRNA was determined using the RT2 Profiler PCR Array (Qiagen, Germany). A PCR master mixture, consisting of 2x RT2 SYBR Green Mastermix, cDNA synthesis product, and RNase-free water, was prepared in a 5 mL tube at room temperature. Twenty-five microliters (25 µL) of the PCR mixture were dispensed into each well of the PCR array plate and sealed using optical adhesive film. The TfR primer (Catalogue number: PPR067324) and Actin B were pre-coated on the plate. qPCR was run on the StepOnePlusTM Real-Time PCR System (Applied Biosystems, USA) with an initial denaturation at 95ºC for 10 minutes, followed by 40 cycles of denaturation at 95˚C for 15 seconds and annealing at 60°C for 1 minute.

2.5 Immunohistochemistry (IHC)

The small intestine (5 cm) was stored in 10% neutral formalin solution for 12 hours before the tissue was fixed in the Leica TP1020 Automated Tissue Processor (Leica, Germany), where it was submerged in a series of alcohol and toluene. The fixed tissue was then embedded in the paraffin block, and a 3 µm ribbon was sectioned and floated on 40°C water before being transferred onto a glass slide. Following deparaffinization and rehydration using xylene and alcohol, the tissue was immersed in 10% neutral buffered formalin for 30 minutes and incubated with a peroxidase block for 10 minutes and then rinsed with PBST. The slide was incubated in citrate buffer (pH 6) at 95°C for 1 hour in a water bath, then rinsed with PBST. A protein block was applied to the slide for 10 minutes at room temperature, followed by rinsing with PBST. The tissue was then incubated in mouse anti-transferrin receptor antibody (Abcam, UK) at a 1:6000 dilution overnight at 4°C. After rinsing with PBST, the tissue was incubated in the Dako EnVision+ System-HRP Labelled Polymer Anti-Rabbit (Agilent, USA) for 30 minutes at room temperature. Following rinsing with PBST, DAB chromogen was applied to the slide for 5 minutes, and then it was counterstained with hematoxylin for 1 minute and soaked in bluing reagent for 1 minute. The slide was then dehydrated in a series of graded alcohol and xylene before a coverslip was mounted onto the slide. The immunoreactivity was analyzed under the Olympus BX51 microscope, and digital images were captured with the Olympus C7070 camera. Cellular localization and staining intensity were determined and scored independently by two pathologists. The intensity was graded as 0 (no expression), 1 (weak), 2 (moderate), and 3 (strong).

2.6 Statistical analysis

The results obtained were expressed as mean ± SEM. Statistical analyses were performed using SPSS 23.0 software (SPSS Inc., USA). Significant difference pre- and post-intervention were determined using Student's T-Test while significant difference between groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. For immunohistochemistry (IHC) the Mann-Whitney test was used, with significance accepted at $p<0.05$.

3. RESULTS

3.1 Hb, serum iron and Tf saturation level

The Hb, serum iron and Tf saturation levels in the normal control and rats fed an iron-deficient diet to induce IDA are presented in Table 1. Generally, the Hb, serum iron, and Tf saturation levels in the anemic rats were significantly lower than those in the normal control. Feeding an iron-deficient diet to these rats for two weeks resulted in them becoming anemic. Date palm and goat milk, both singly and in combination, were supplemented to the IDA rats for 4 weeks. Hb, serum iron, and Tf saturation levels all showed a significant increase ($p<0.05$) compared to the normal control post-intervention (Table 2). With the exception of the negative control, the supplemented and positive control rats showed Hb, serum iron, and Tf saturation levels reaching normal values significantly. Normalization of the values in the IDA rats supplemented with date palm and goat milk seemed to be similar to that in the positive control. The values for the positive control and intervention groups were not significantly different. Tf saturation levels were significantly altered in the supplemented IDA rats, with the levels in date palm and goat milk, both singly and in combination, being significantly higher than those in the positive control.

Table 1. Hb, serum iron and transferrin saturation after anemia induction.

Parameters	Normal control	Neg Control	Pos Control	DP	GM	DPGM
Hb (g/dL)	13.50 ± 0.12	8.58 ± 2.43[a]	10.35 ± 0.51[a]	10.30 ± 0.65[a]	10.03 ± 0.60[a]	10.35 ± 1.43[a]
Serum Fe (µmol/L)	34.15 ± 4.54	11.53 ± 1.59[a]	6.45 ± 7.14[a]	8.53 ± 2.12[a]	11.30 ± 5.61[a]	7.23 ± 4.87[a]
Tf (%)	53.00 ± 4.38	20.50 ± 3.07[a]	11.25 ± 13.33[a]	14.50 ± 3.51[a]	25.00 ± 10.80[a]	13.75 ± 10.37[a]

All parameters are expressed as mean ± SEM
[a] within the same row indicates significant differences ($p<0.05$) as compared to normal control
DP: Date palm; GM: Goat milk; DPGM: Date palm and goat milk.

Table 2. Hb, serum iron and transferrin saturation after intervention with date palm and goat milk.

Parameters	Normal control	Neg Control	Pos Control	DP	GM	DPGM
Hb (g/dL)	16.18 ± 0.49[*]	6.68 ± 3.09[a]	16.18 ± 0.82[*, b]	15.55 ± 0.62[*,b]	13.85 ± 2.77[b]	15.58 ± 0.33[*,b]
Serum Fe (µmol/L)	39.90 ± 2.79	3.13 ± 0.74[*,a]	29.35 ± 2.33[*,a,b]	31.95 ± 5.26[*,b]	30.48 ± 3.59[*,a,b]	31.93 ± 2.95[*,b]
Tf (%)	89.25± 6.18[*]	6.75 ± 1.49[*,a]	49.50 ± 3.70[*,a,b]	61.50 ± 10.97[*,a,b]	76.75 ± 8.42[*,b]	60.25 ± 6.24[*,a,b]

All parameters are expressed as mean ± SEM
* indicates significant differences ($p<0.05$) pre- and post-intervention with date palm and goat milk
[a] within the same row indicates significant differences ($p<0.05$) as compared to normal control
[b] within the same row indicates significant differences ($p<0.05$) as compared to negative control
DP: Date palm; GM: Goat milk; DPGM: Date palm and goat milk.

3.2 *Expression of TfR mRNA following intervention with date palm and goat milk*

Figure 1 shows TfR mRNA expression in treated rats. The negative control showed the highest TfR expression compared to the normal control, positive control, and the supplemented rats (p<0.05). The positive control and date palm and goat milk-supplemented rats showed attenuated TfR expression compared to that of the normal control. The presence of iron in the diet somehow diminished TfR expression (p<0.05) in the supplemented rats compared to the normal control, while an iron-deficient diet markedly increased TfR expression (p<0.05). No significant difference in TfR expression was observed between the positive control and supplemented rats.

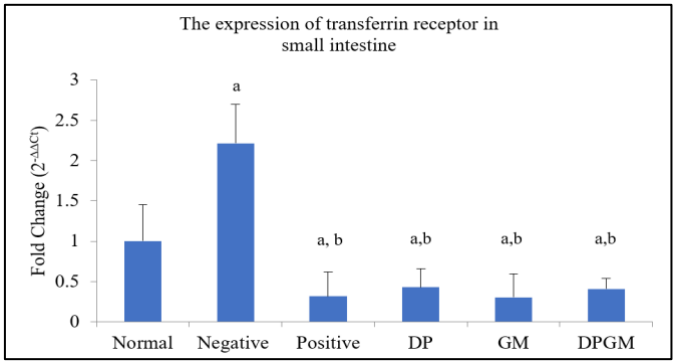

Fig. 1. TfR mRNA expression in the small intestine of IDA-induced rats after intervention with date palm and goat milk. All parameters are expressed as the ratio of transferrin receptor/Rps18. Data represent mean ± SEM. a and b indicate significant differences (p<0.05) when compared to the normal control and negative control, respectively. DP: Date palm; GM: Goat milk; DPGM: Date palm and goat milk.

3.3 *Localization of TfR protein in small intestine*

Small intestines were stained for TfR protein reactivity (Figure 2). In normal condition, TfR is produced in the epithelial cells of the crypts and the villi in the small intestines. As the epithelial cells migrate apically towards the villus tip, the protein density decreases, as shown in the normal control (Figure A). The reactivity was mostly cytoplasmic, with no stain observed on the brush border membrane of the villi and goblet cells (Figure B). In the negative control, significantly higher staining intensity was observed (p<0.05) compared to the normal control (Figure C). Staining intensity was also evenly distributed on both apical and basolateral sides of the epithelium villi. Positive reactivity was also observed on the brush border membrane of the enterocyte (Figure D). The positive control small intestines retained a similar expression to the normal control, with staining intensity decreasing from the crypt's region to the villus tip (Figure E). No staining was observed on the brush border region of the villi. All intervention groups exhibited a similar trend of TfR protein localisation as the normal control (Figure G-L).

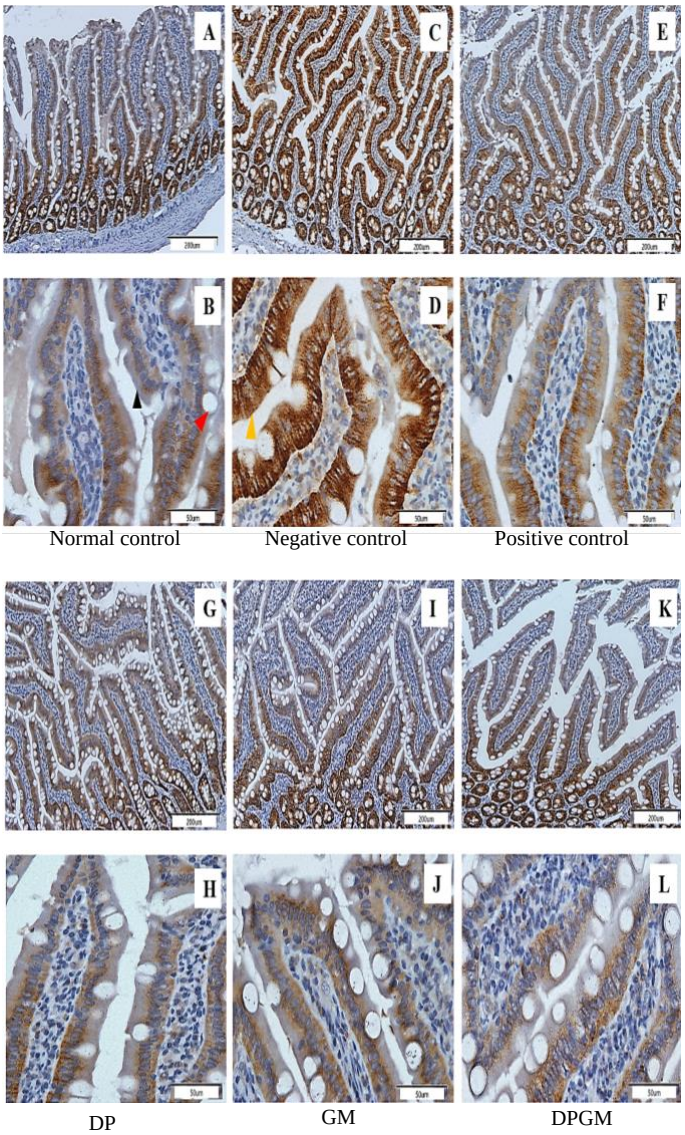

Fig. 2. Immunohistochemical localisation of TfR protein in the small intestine of the rat. Normal control showed TfR protein expression within the epithelial cells of the crypts and the enterocytes villi. Staining intensity decreased while epithelial cells migrated toward the villus tip (Figure A). No staining in the mucus-secreting goblet cells (red arrowhead in Figure B) and brush border membrane (black arrowhead in Figure B). Negative control showed prominent staining on crypts and villi with strong reactivity on both apical and basolateral sides of the epithelial villi (Figure C). Brush border membrane also stained positive (yellow arrowhead in Figure D). No staining in the goblet cell. Positive control, date palm, goat milk, date palm and goat milk retained a similar expression of TfR as in normal control. All images were captured at X20 and X40 magnification. DP: Date palm; GM: Goat milk; DPGM: Date palm and goat milk.

Table 1. Semiquantitative analysis of immunoreactivity of TfR in the small intestine of IDA induced rats supplemeneted with date palm anad goat milk

Protein	Normal control	Negative control	Positive control	DP	GM	DPGM
TfR	1.75 (1.75)	2.75 (3.00) *	2.50 (2.75)	2.25 (2.25)	2.25 (2.25)	1.88 (1.75)

The mean and median of each group is presented, mean (median).* Significant different when compared to normal control (p <0.05). DP: Date palm; GM: Goat milk; DPGM: Date palm and goat milk.

4. DISCUSSIONS

IDA stands as one of the most prevalent nutritional deficiencies globally, characterized by insufficient iron levels, resulting in a lack of healthy red blood cells and reduced hemoglobin levels, impeding the transport of oxygen throughout the body. In our study, we observed a downregulation of Hb and serum iron levels during iron deficiency, with serum iron and Tf saturation falling significantly below the normal range.

Additionally, the expression of the TfR mRNA in the small intestine was significantly up-regulated during iron deficiency, consistent with previous research finding [24]–[26]. Iron deficiency also led to a significant increase in TfR protein expression within the small intestine. Positive immunoreactivity was detected on the intestinal villi, exhibiting intense reactivity on both the apical and basolateral membranes of the epithelial lining when compared to the normal control. Positive immunoreactivity was also observed on the brush border membrane of the villi.

Following the administration of date palm and goat milk, improvement in IDA were evident in IDA-induces rats, as indicated by diagnostic iron parameters such as Hb level, serum iron and Tf saturation level. The expression of TfR mRNA protein was normalized, returning to normal level. The groups supplemented with date palm and goat milk exhibited a pattern of TfR expression similar to that of the normal control. TfR protein expression was concentrated solely on the basolateral membrane of epithelial cells, with no reactivity on the brush border membrane of the villi. In normal control small intestine, TfR protein expression was detected in the crypts and villi of the enterocyte epithelial cell. Its intensity decreased as epithelial cells migrated apically toward the villus tips, displaying a diffuse intracellular cytoplasmic expression, aligning with previous studies [27], [28]. The immunohistochemistry findings were in line with the gene expression result, in which TfR mRNA expression in the small intestine upsurged during iron deficiency and the supplementation of date palm and goat milk normalized the expression level of TfR with that of the normal control. TfR, a membrane glycoprotein, mediates cellular iron uptake from plasma glycoprotein Tf. Its soluble form is produced by proteolysis of the membrane TfR, and its concentration is proportional to the amount of TfR present in cells. TfR expression is inversely proportional to cellular iron levels. When cellular iron is scarce, TfR expression increases, whereas in the presence of excess iron, TfR expression decreases. TfR is found on the surface of nearly all cells that require iron for proliferation, and it is particularly abundant in erythroid cells, placenta, and the liver. Erythropoiesis, the process of red blood cell formation, is the most iron-demanding process as it is crucial for producing hemoglobin needed to transport oxygen throughout the body. A prior study demonstrated that selectively inactivating the TfR gene in murine intestinal epithelial cells resulted in impaired proliferation of intestinal epithelial cell progenitors, ultimately leading to early death [8]. The study suggested that TfR likely plays a role in supplying iron to developing epithelial cells in the intestinal crypts, which is essential for maintaining intestinal integrity. Given the rapid turnover of the small intestine epithelia (2.8 days for rat and 3.48 days in human) [29], a considerable amount of iron is also required to support new cell growth. Thus, in iron deficient condition, TfR expression in small intestine was significantly up-regulated, in order to encourage more iron to be taken up from dietary absorption to compensate for erythropoiesis activity and also to maintain the intestinal integrity. This study also showed that TfR level was also inversely proportional with Hb, serum iron level and Tf saturation level. A study showed that serum TfR increased in IDA children and showed a negative correlation with Hb, MCV, serum iron and Tf saturation [30].

Date palm is rich in carbohydrate, dietary fibres, vitamins, minerals, fatty acids, and phytochemicals, and is suitable for regular daily consumption [31]. Date palm has a significant amount of iron, which, when combined with ascorbic acid, increases iron bioavailability. Ascorbic acid is vital in iron metabolism as it enhances iron uptake into the enterocytes by reducing Fe^{3+} to Fe^{2+} during iron acquisition in the small intestine. Ascorbic acids were also reported to chelate iron and stimulate the synthesis of iron store ferritin [32]. Date palm has been documented to bring about tremendous health benefits protecting against infection, toxicity, cancer, inflammation, heart disease, liver disease, hypertension, diabetes, constipation and anemia [33]. Goat milk, on the other hand, is increasing in popularity as a functional food due to its nutritional properties and lower allergenicity in comparison to cow milk [34]. Compared to cow milk, goat milk has a smaller fat globule size and contains a higher concentration of medium-chain triglycerides (MCT), resulting in better digestibility, more efficient lipid metabolism and softer texture [35]. Numerous prior studies have shown that goat milk provides a better use of iron, which minimizes possible interaction between iron and other minerals and hence, improve the bioavailability of copper, zinc, magnesium, phosphorus and selenium in animal models [36]–[38]. A study revealed that, despite the high calcium content in calcium-supplemented goat milk, its consumption had no deleterious impacts on iron absorption in IDA rat [39]. Similar finding was reported in which they discovered that not only does calcium-fortified goat milk favour iron deficiency anaemia recovery, but it also increases the bioavailability of copper, a mineral essential for erythropoiesis [40]. Naturally occurring oligosaccharides in goat milk also exhibit prebiotic potential that promotes the growth of beneficial gut microbiota and at the same time possess anti-infection property [34]. Bioactive peptides from goat milk proteins are shown to exhibits numerous physiological and functional effects in the body such as antihypertensive, antioxidative, antithrombotic, opioid, hypocholesterolaemia and immunomodulatory activities [41].

Our results showed that post iron repletion with date palm and goat milk in IDA-induced rat significantly improve IDA condition by normalizing Hb, serum iron and Tf saturation level. Decrease in TfR mRNA and protein expression in the small intestine was also observed as iron availability increase, improving overall IDA condition.

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REFERENCES

[1] NHMS, "National Health and Mobidity Survey 2019. Non-communicable disease, healthcare demand, and health literacy; Key Findings," 2019.

[2] WHO, "Anaemia in Women and Children: WHO Global Anaemia Estimates, 2021 Edition.," World Health Organization, 2021. [Online]. Available: https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children. [Accessed: 02-Sep-2022].

[3] S. Safiri et al., "Burden of anemia and its underlying causes in 204 countries and territories, 1990–2019: results from the Global Burden of Disease Study 2019," J. Hematol. Oncol., vol. 14, no. 1, pp. 1–16, 2021.

[4] M. D. Cappellini, K. M. Musallam, and A. T. Taher, "Iron deficiency anaemia revisited," J. Intern. Med., vol. 287, no. 2, pp. 153–170, 2020.

[5] D. Galaris, A. Barbouti, and K. Pantopoulos, "Iron homeostasis and oxidative stress: An intimate relationship," Biochim. Biophys. Acta - Mol. Cell Res., vol. 1866, no. 12, p. 118535, 2019.

[6] A. C. S. Vogt, T. Arsiwala, M. Mohsen, M. Vogel, V. Manolova, and M. F. Bachmann, "On iron metabolism and its regulation," Int. J. Mol. Sci., vol. 22, no. 9, pp. 1–17, 2021.

[7] E. Gammella, P. Buratti, G. Cairo, and S. Recalcati, "The transferrin receptor: The cellular iron gate," Metallomics, vol. 9, no. 10, pp. 1367–1375, 2017.

[8] A. C. Chen, A. Donovan, R. Ned-Sykes, and N. C. Andrews, "Noncanonical role of transferrin receptor 1 is essential for intestinal homeostasis," Proc. Natl. Acad. Sci. U. S. A., vol. 112, no. 37, pp. 11714–11719, 2015.

[9] S. Recalcati, E. Gammella, P. Buratti, and G. Cairo, "Molecular Regulation of Cellular Iron Balance," IUBMB Life, vol. 69, no. 6, pp. 389–398, 2017.

[10] Division of Family Health Development, "Perinatal Care Manual 4th Edition," 2020.

[11] R. Abd Rahman, I. B. Idris, Z. M. Isa, R. A. Rahman, and Z. A. Mahdy, "The Prevalence and Risk Factors of Iron Deficiency Anemia Among Pregnant Women in Malaysia: A Systematic Review," Front. Nutr., vol. 9, no. April, pp. 1–9, 2022.

[12] N. A. Kadir, N. A. A. Rahim, E. Mangantig, N. A. Z. N. Lah, and A. H. Ahmad, "Knowledge of oral iron consumption among pregnant women at Hospital Universiti Sains Malaysia," Malaysian J. Med. Heal. Sci., vol. 17, no. 9, pp. 107–117, 2021.

[13] N. A. A. Daud, S. A. Mukhlef, and F. A. Aziz, "Awareness, attitude and behaviour related to the intake of dietary supplements among Malaysian pregnant women," Malaysian J. Med. Heal. Sci., vol. 16, no. 4, pp. 81–88, 2020.

[14] S. García López, A. Rubio, V. Poza, Bermejo, and S. García López, "Optimal management of iron deficiency anemia due to poor dietary intake," Int. J. Gen. Med., vol. 1, no. 4, pp. 741–750, 2011.

[15] J. P. Chouraqui, "Dietary Approaches to Iron Deficiency Prevention in Childhood—A Critical Public Health Issue," Nutrients, vol. 14, no. 8, pp. 1–24, 2022.

[16] A. Heffernan, C. Evans, M. Holmes, and J. B. Moore, "The Regulation of Dietary Iron Bioavailability by Vitamin C: A Systematic Review and Meta-Analysis," Proc. Nutr. Soc., vol. 76, no. OCE4, p. 4433, 2017.

[17] N. A. Majid, Z. Zainol, N. A. Shamaan, N. A. Hamid, N. Roslan, and N. F. Zulkifli, "Date palm and goat milk improve haematological parameters and availability of functional iron in iron deficient rats," Malaysian J. Med. Heal. Sci., vol. 16, no. 3, pp. 52–59, 2020.

[18] M. M. Abdelsalam, M. M. Zeitoun, M. A. Ateah, A. Al-Hassan, and A. M. Abdel-Salam, "Impact of probiotic fermented milk, palm date extract and their mixture supplementation on neonatal traits and hematological parameters of late pregnant najdi ewes," International Journal of Biological Chemistry, vol. 8, no. 1. pp. 37–47, 2014.

[19] A. T. H. Zen, D. Pertiwi, and Chodidjah, "Pengaruh Pemberian Sari Kurma (Phoenix dactylifera) terhadap Kadar Hemoglobin Studi Eksperimental pada Tikus Putih Jantan Galur Wistar yang Diberi Diet Rendah Zat Besi (Fe)," Sains Med., vol. 5, no. 1, pp. 17–19, 2013.

[20] S. N. Onuh, E. O. Ukaejiofo, P. . Achukwu, S. A. Ufelle, C. N. Okwuosa, and C. J. Chukwuka, "Haemopoietic activity and effect of Crude Fruit Extract of Phoenix dactylifera on Peripheral Blood Parameters," Int. J. Biol. Med. Res., vol. 3, no. 1, pp. 1720–1723, 2012.

[21] J. Díaz-Castro, M. J. M. Alférez, I. López-Aliaga, T. Nestares, and M. S. Campos, "Effect of calcium-fortified milk-rich diets (either goat's or cow's milk) on copper bioavailability in iron-deficient anemia," Dairy Sci. Technol., vol. 91, no. 2, pp. 203–212, 2011.

[22] M. J. M. Alférez et al., "Dietary goat milk improves iron bioavailability in rats with induced ferropenic anaemia in comparison with cow milk," Int. Dairy J., vol. 16, no. 7, pp. 813–821, 2006.

[23] J. Diaz-Castro, M. J. M. Alférez, J. Moreno-Fernandez, and I. López-Aliaga, Influence of Goat Milk on Bone and Mineral Metabolism During Iron Deficiency Recovery. Elsevier Inc., 2017.

[24] Y. Q. Li, B. Bai, Q. Q. Zheng, H. Yan, and G. H. Zhuang, "Quantitative Study of Iron Metabolism-related Genes Expression in Rat," Biomed. Environ. Sci., vol. 26, no. 10, pp. 808–819, 2013.

[25] F. Dupic et al., "Duodenal mRNA expression of iron related genes in response to iron loading and iron deficiency in four strains of mice," Gut, vol. 51, pp. 648–653, 2002.

[26] A. Mckie, K. Raja, T. Peters, F. Farzaneh, and R. Simpson, "Expression metabolism of genes involved in iron in mouse intestine," Am. J. Physiol., vol. 271, pp. G772–G779, 1996.

[27] S. Deaglio et al., "Structural , functional , and tissue distribution analysis of human transferrin receptor-2 by murine monoclonal antibodies and a polyclonal antiserum," Blood, vol. 100, no. 10, pp. 3782–3789, 2002.

[28] V. L. Kolachala, B. Sesikeran, and K. M. Nair, "Evidence for a sequential transfer or iron amongst ferritin, transferrin and transferrin receptor during duodenal absorption of iron in rat and human," World J. Gastroenterol., vol. 13, no. 7, pp. 1042–1052, 2007.

[29] A. Darwich, U. Aslam, D. Ashcroft, and A. Rostami-Hodjegan, "Meta-analysis of the turnover of intestinal epithelia in preclinical animal species and humans," Drug Metab. Dispos., vol. 42, no. 12, pp. 2016–2022, 2014.

[30] S. H. Yoon, D. S. Kim, S. T. Yu, S. R. Shin, and D. Y. Choi, "The usefulness of soluble transferrin receptor in the diagnosis and treatment of iron deficiency anemia in children," Korean J. Pediatr., vol. 58, no. 1, pp. 15–19, 2015.

[31] A. T. Idowu, O. O. Igiehon, A. E. Adekoya, and S. Idowu, "Dates palm fruits: A review of their nutritional components, bioactivities and functional food applications," AIMS Agric. Food, vol. 5, no. 4, pp. 734–755, 2020.

[32] D. J. R. Lane and D. R. Richardson, "The active role of vitamin C in mammalian iron metabolism: Much more than just enhanced iron absorption!," Free Radic. Biol. Med., vol. 75, pp. 69–83, 2014.

[33] M. B. Mirza, F. Q. Syed, F. Khan, A. I. Elkady, A. M. Al-Attar, and K. R. Hakeem, "Ajwa dates: A highly nutritive fruit with the impending therapeutic application," Plant Hum. Heal. Pharmacol. Ther. Uses, vol. 3, pp. 209–230, 2019.

[34] M. J. R. Lima, E. Teixeira-Lemos, J. Oliveira, L. P. Teixeira-Lemos, A. M. C. Monteiro, and J. M. Costa, "Nutritional and Health Profile of Goat Products: Focus on Health Benefits of Goat Milk," in Goat Science, S. Kukovics, Ed. Portugal: Licensee IntechOpen, 2018, pp. 189–232.

[35] N. Turkmen, "The Nutritional Value and Health Benefits of Goat Milk Components," in Nutrients in Dairy and Their Implications for Health and Disease, W. Ronald, R. Collier, and V. Preedy, Eds. Elsevier Inc., 2017, pp. 441–449.

[36] M. S. Campos, I. López-Aliaga, M. J. M. Alférez, T. Nestares, and M. Barrionuevo, "Effects of goats' or cows' milks on nutritive utilization of calcium and phosphorus in rats with intestinal resection," Br. J. Nutr., vol. 90, no. 1, pp. 61–67, 2003.

[37] M. Barrionuevo, M. J. M. Alferez, I. Lopez Aliaga, M. R. Sanz Sampelayo, and M. S. Campos, "Beneficial Effect of Goat Milk on Nutritive Utilization of Iron and Copper in Malabsorption Syndrome," J. Dairy Sci., vol. 85, no. 3, pp. 657–664, 2002.

[38] M. Barrionuevo, I. López Aliaga, M. J. M. Alférez, E. Mesa, T. Nestáres, and M. Sánchez Campos, "Beneficial effect of goat milk on

bioavailability of cooper, zinc and selenium in rats," J. Physiol. Biochem., vol. 59, no. 2, pp. 111–118, 2003.

[39] I. López-Aliaga, J. Díaz-Castro, T. Nestares, M. J. M. Alférez, and M. S. Campos, "Calcium-supplemented goat milk does not interfere with iron absorption in rats with anaemia induced by dietary iron depletion," Food Chem., vol. 113, no. 3, pp. 839–841, 2009.

[40] J. Díaz-Castro, M. J. M. Alférez, I. López-Aliaga, T. Nestares, and M. S. Campos, "Effect of calcium-fortified milk-rich diets (either goat's or cow's milk) on copper bioavailability in iron-deficient anemia," Dairy Sci. Technol., vol. 91, no. 2, pp. 203–212, 2011.

[41] Y. W. Park, "Nutritional and Chemical Composition of Goat Milk," Handb. Milk Non-Bovine Mammals, Second Ed., no. 119, pp. 34–58, 2017.