ORIGINAL ARTICLE

Date Palm and Goat Milk Improve Haematological Parameters and Availability of Functional Iron in Iron Deficient Rats

Nurainna Abd Majid, Zuriani Zainol, Nor Aripin Shamaan, Nazefah Abd Hamid, Nuruliza Roslan, Noor Fadzilah Zulkifli

Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, Level 13, Menara B, Persiaran MPAJ, 55100 Pandan Indah, Malaysia

ABSTRACT

Introduction: Iron deficiency anaemia (IDA) is endemic especially in the under-developed and developing countries and is a major public health concern. Improving nutrition is one of the ways to alleviate this condition. Consumption of locally available and affordable food such as date palm and goat milk which are rich in iron is one of the ways to overcome IDA. This study is aimed at evaluating the effect of date palm and goat milk supplementation on haematological parameters and iron bioavailability in IDA rats. **Methods:** 24 male Wistar rats were randomly divided into normal control and IDA group. The normal control was fed with normal diet and water ad libitum while the IDA group were fed on iron-deficient diet for two weeks to induce iron deficiency. The IDA rats were further divided into subgroups; each being supplemented with date palm, goat milk, a combination of date palm and goat milk, and ferrous fumarate as positive control. Blood were collected after 28 days for haematological parameters and iron profile determination. Iron bioavailability was assessed using the haemoglobin regeneration efficiency (HRE) index. Data was analysed by Student T Test and ANOVA using SPSS 23.0 software with p value < 0.05 considered as statistically significant. **Results:** Supplementation of date palm and goat milk for 28 days significantly improved Hb, RBC, PCV, MCV, MCH, serum iron and transferrin saturation ($p<0.05$) in all treatment groups compared to normal control. The iron bioavailability of date palm and goat milk supplemented rats was similar to that of the positive control. **Conclusion:** Supplementation of date palm and goat milk in IDA rats improves haematological parameters and iron bioavailability.

Keywords: Date palm, Goat milk, Iron bioavailability, Iron deficiency anaemia, Rats

Corresponding Author:
Noor Fadzilah Zulkifli, MPath
Email: nfadzilahz@usim.edu.my
Tel: +603-42892400

INTRODUCTION

Iron deficiency anaemia (IDA) is a pathological condition in which the decreased level of total body iron is accompanied with anaemia. The reported prevalence of IDA in Malaysia is approximately 32% in preschool children, 20% in non-pregnant women at child bearing age and 27% in pregnant women (1). As symptoms of IDA can be subclinical, many patients were left undiagnosed. Weakness, fatigue, poor concentration and work performances are among common subtle symptoms associated with IDA due to the low oxygen bioavailability in body tissues (2). IDA is caused by either poor intake of dietary iron or poor dietary iron bioavailability. Iron bioavailability is defined as the fraction of ingested iron that is absorbed and subsequently utilized for normal physiological functions (3). Total iron bioavailability is the extent to which heme and non-heme iron is absorbed from the diet and used for normal body functions. Heme iron absorption is more constant and uniform (>40%) than non-heme iron. Iron absorption relies on the balance between absorption enhancers, inhibitors and the individual body iron status (4). Ascorbic acid and animal tissues are among enhancers of iron absorption while polyphenol, calcium and phytic acid are inhibitors of non-heme iron absorption (5). Currently, IDA is endemic globally due to malnutrition and poor nutrition. Besides the iron content in the food, iron bioavailability in a food also plays important role in the management of IDA.

Date palm fruit (*Phoenix dactylifera*) is one of the oldest plant foods having numerous nutritional, environmental, economic, and ornamental benefits. Approximately 5000 date palm varieties are grown in different regions of the world (6). Date palm is a high energy food with its sugar content varying between 33.2 to 74.3% in the form of digestible sugars such as glucose, fructose, and sucrose. It is also composed of protein, dietary fibre, minerals, essential amino acid and a low level of fat. Other components include vitamins (A, B1, B2, B3, and C), carotenoids, and flavonoids (7). Numerous nutritional benefits make it a suitable choice as a supplement for

maintaining overall wellbeing. Date palm possesses anti-anaemic properties attributable to the high level of iron and vitamin C. It contains approximately 0.15 - 0.5 mg/100g of iron (8,9) and 2.4 - 17.5mg/100g of ascorbic acid (10). Vitamin C also known as ascorbic acid acts as reductant and cofactor in iron haemostasis. It enhances the absorption of nonheme iron in the small intestine, stimulates the synthesis of ferritin, inhibits lysosomal ferritin degradation and reduces cellular iron efflux. Recent evidence also indicates that ascorbate is a novel modulator in the transferrin iron uptake pathway that provides iron for cellular demands and erythropoiesis under physiological condition (11). Ascorbic acid has been reported to enhance the absorption of non-heme iron, thus improving the anaemia status (12). As iron is vital in erythropoiesis, the consumption of date palm may assist in alleviating IDA. Numerous studies had shown that date palm consumption is able to increase haematological parameters in anaemic subjects (13,14).

Goats are among the earliest domesticated animals, being utilized as a source of food, clothing and building materials. Nearly all goat milk production come from *Capra hircus* species. On average, goat milk contains 13.2% total solids, made up of 4.5% fat, 3.6% protein, 4.3% lactose and 0.8% minerals (15). Goat milk was reported to produce a greater nutritive use of iron when compared to cow's milk (16) . Milk and other dairy products from the cow which are rich in calcium are known to interfere with iron absorption (17). Studies had shown that although the iron content of goat milk and cow's milk are similar, with approximately 0.05mg for every 100g, the iron bioavailability of goat milk was much greater than cow's milk (18). Moreover, compared with cow's milk, goat milk has better digestibility, alkalinity, buffering capacity and overall dietary characteristics. Goat milk has smaller fat globules and greater proportion of medium-chain triglycerides (MCT), making it more digestible and easily absorbed in the small intestine (15). Goat milk has low level of αs1-casein that causes allergenicity in milk (19) and other studies have revealed that it possesses antibacterial, immunomodulatory and antihypertensive activities (20). Food based approaches represent the most desirable and sustainable way in preventing malnutrition worldwide. Due to its excellent nutritional content, both date palm and goat milk has potential as alternatives in managing IDA. Thus, the purpose of this study is to investigate iron bioavailability in date palm and goat milk and their effect on haematological parameters and iron profile in rats induced with IDA.

MATERIALS AND METHODS

Date palm fruit (*Phoenix dactylifera* L, Ajwa var.) and fresh goat milk (*Capra aegagrus hircus*, Saanen breed) were purchased from a local supplier.

Nutritional Analysis

The Ajwa dates and goat milk were sent to Permulab Sdn. Bhd., Petaling Jaya, Selangor for nutritional analysis using High Performance Liquid Chromatography (HPLC) and Inductively Coupled Plasma Emission Spectrometry (ICP-OES).

Preparation of Ajwa date and goat milk

Phoenix dactylifera raw solution was prepared according to method by Al-Qarawi *et al.*,(21), by homogenising dried date palm flesh with distilled water (1/3, w/v) using a domestic hand blender. The prepared date palm solution and goat milk were stored at -4°C and -20°C respectively, before use.

Experimental animal

A total of 24 male Wistar rats at 4 weeks of age were purchased from Animal House, Universiti Kebangsaan Malaysia, Kuala Lumpur. The rats were housed in standard rat cages in an ambient condition with temperature of 25°C, and humidity of 60–70%, in a 12 light/12 dark cycle. The rats were given rodent pellet and distilled water *ad libitum*. After acclimatization for 7 days, the rats were randomly divided into 2 groups. Normal control group (n=4) were given the ordinary rat pellet throughout the study whereas the IDA model group (n=20) were given iron deficient pellet (2mg/kg) (Harlan, USA) and distilled water *ad libitum* for two weeks to induce iron deficiency. Haemoglobin (Hb) levels were monitored weekly and IDA was confirmed when Hb values is less than 11.5 g/dL (22). The Hb level was measured using DiaSpect Hemoglobin TM Analyzer (DiaSpect, Germany). The IDA model group with similar baseline Hb values were divided randomly into 5 groups (n=4 each group). They were supplemented with one of these protocol; iron tablet of 20.5mg/kg as ferrous fumarate tablet (Hovid, Malaysia) which acts as positive control, date palm (4300mg/kg) (DP), goat milk (25.7ml/kg) (M), and combination of date palm and goat milk (4300mg/kg and 25.7ml/kg fresh goat milk) (DM). The negative control group were given iron deficient pellet without any supplement. The dosage was calculated based on Animal Equivalent Dosage (AED) formula (23). The supplements were administered every morning for 28 days by oral gavage. Blood sampling was collected by lateral tail vein in the rats at the beginning of the study and after 28 days for haematological parameters and iron profile. The handling and use of these laboratory animals were approved by the Animal Research Ethics Committee of Universiti Sains Islam Malaysia, Kuala Lumpur (USIM/AEC/AUP/2016).

Iron bioavailability

The iron bioavailability was calculated as haemoglobin regeneration efficiency (HRE). It provides an estimate of the percentage of ingested iron that is absorbed. HRE was calculated using this formula (24):

$$(\text{HRE \%}) = \frac{[\text{Hb Fe Final - Hb Fe initial x 100}]}{\text{Total Fe intake}}$$

Hb Fe is derived from the formula below:

$$\text{Hb Fe} = \text{BW} \times 0.067 \times \text{Hb} \times 3.35$$

Hb Fe is the total body haemoglobin iron (mg); Hb is the concentration of haemoglobin (g/dL); BW is the body weight (kg); 0.067 refers to volume of blood (in litre) per kilogram body weight (L/kg); and 3.35 refers to weight of iron (in mg) per gram haemoglobin (mg/g).

Haematological and biochemical analysis

The collected blood was analysed for haematological parameters using Sysmex XE – 2100 Automated Hematology Analyser (Sysmex, Japan) while iron profile was analysed by Advia 2400 Chemistry System (Siemens, Germany).

Statistical analysis

The results obtained were expressed as mean ± SD. All statistical analyses were performed using SPSS 23.0 software (SPSS Inc, Chicago, USA). Significant difference pre and post treatment was determined using Student's T-Test while significant different between group was determined by one-way analysis of variance (ANOVA) followed by Tukey Post Hoc test with $p < 0.05$ were considered significantly different.

RESULTS

Chemical composition of Ajwa date and goat milk

The chemical composition of both Ajwa dates and goat milk were shown in Table I. The dried date flesh contains a high level of sugar at 31.6g glucose and 29.5g fructose per 100g, respectively. It also contains iron (10.7mg/kg), calcium (536mg/kg), magnesium (418mg/kg), manganese (5.23mg/kg) and 9.80mg of ascorbic acid.

Goat milk contained 3.38g per 100 mL lactose while glucose and fructose were not present. Iron (0.09 mg per 100 mL), magnesium (0.10mg per 100 mL) and calcium (72.2mg per 100 mL) were detected. Ascorbic acid and manganese, however, were not detected in goat milk.

Iron bioavailability

All treatment groups showed HRE ranging from 4.58% to 5.58% and showed significant difference ($p<0.05$) when compared to negative control. The negative control group has significantly lower final haemoglobin iron content compared to other groups giving a high HRE percentage of 21.7% (Table II).

Haematological and biochemical analysis

The haematological parameters and biochemical analysis of rats after anaemic induction were shown in Table III. Decreased level in Red Blood Cell (RBC) count, Hb, Packed Cell Volume (PCV), serum iron and transferrin saturation were observed in all treatment groups when compared to normal groups. After supplementation of 4 weeks, the Hb level in the IDA induced rats was significantly improved ($p<0.05$) (Table IV). Among the supplemented groups, rats supplemented on goat milk showed lower Hb value (13.85g/dL) compared with the date palm and combination group. A significant increased ($p < 0.05$) was also found in RBC, PCV, serum iron and transferrin saturation level after supplementation of date palm and goat milk. Transferrin saturation in all treatment groups increased

Table I: Chemical composition of Ajwa date and goat milk

Ingredient	Date Palm	Goat Milk
Sugars	**(g/100g)**	**(g/100mL)**
Glucose	31.60	nd
Fructose	29.50	nd
Lactose	nd	3.38
Mineral	**(mg/kg)**	**(mg/100mL)**
Iron	10.70	0.09
Manganese	5.23	nd
Zinc	2.39	0.10
Calcium	536	72.2
Magnesium	418	10
Vitamin	**(mg/100g)**	**(mg/100mL)**
Ascorbic Acid	9.80	nd

nd: not determined

Table II: Haemoglobin regeneration efficiency (HRE) in control and rats treated with date palm and goat milk after 28 days treatment

Group	Normal Control	Negative Control	Positive Control	Date palm (DP)	Goat milk (GM)	Date palm and goat milk (DPGM)
Initial body weight (kg)	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.02	0.21 ± 0.00	0.20 ± 0.01	0.22 ± 0.01
Final Body weight (kg)	0.30 ± 0.02	0.28 ± 0.03	0.35 ± 0.02	0.33 ± 0.03	0.32 ± 0.03	0.32 ± 0.03
Initial Hb (g/dL)	13.50 ± 0.24	8.30 ± 0.79	10.35 ± 0.51	10.30 ± 0.65	10.03 ± 0.60	10.35 ± 1.43
Final Hb (g/dL)	16.18 ± 0.84	6.50 ± 0.39	16.18 ± 0.82	15.55 ± 0.62	13.85 ± 2.77	15.58 ± 0.33
Initial Hb Fe (mg)	6.36 ± 0.24	3.91 ± 0.21	4.89 ± 0.29	4.80 ± 0.20	4.51 ± 0.29	5.01 ± 0.42
Final Hb Fe (mg)	10.91 ± 0.84	4.06 ± 0.28	12.71 ± 1.09	11.36 ± 1.36	9.77 ± 1.53	11.29 ± 1.38
HRE (%)	3.7 ± 0.88*	21.7 ± 8.49[a]	5.2 ± 0.38*	5.58 ± 0.82*	4.58 ± 1.84	5.06 ± 1.30*

All values are expressed as mean ± SD, (n=24).
* indicates significant difference ($p< 0.05$) compared with negative control.
[a] indicates significant difference ($p< 0.05$) compared with normal control.

Table III: Hematological parameters and biochemical analysis of rats after 2 weeks anaemia induction

Haematological parameters	Normal control	Negative control	Positive control	Date Palm (DP)	Goat milk (GM)	Date palm + Goat milk (DPGM)
RBC (10^9/L)	6.80 ± 0.34[b]	4.73 ± 0.43[a]	5.85 ± 0.06[a,b]	6.10 ± 0.27[b]	5.80 ± 0.27[a,b]	5.70 ± 0.43[a,b]
Hb (g/dL)	13.50 ± 0.24[b]	8.58 ± 4.85[a]	10.35 ± 0.51[a,b]	10.30 ± 0.65[a,b]	10.03 ± 0.65[a,b]	10.35 ± 1.43[a,b]
PCV (%)	45.50 ± 1.29[b]	29.00 ± 2.45[a]	37.00 ± 2.16[a,b]	36.75 ± 1.50[a,b]	34.00 ± 2.31[a]	32.50 ± 3.70[a]
MCV (fL)	67.00 ± 2.16	61.50 ± 1.73	63.25 ± 3.20	60.00 ± 1.83[a]	58.75± 4.03[a]	57.25 ± 2.99[a]
MCH (pg)	19.75 ± 0.50	18.25 ± 0.96	18.00 ± 0.82[a]	17.75 ± 0.50[a]	18.00 ± 0.82[a]	18.00 ± 0.82[a]
Serum Iron (µmol/L)	34.15 ± 9.07[b]	11.53 ± 3.19[a]	6.45 ± 7.14[a]	8.53 ± 2.12[a]	11.30 ± 5.61[a]	7.23 ± 4.87[a]
Transferrin saturation (%)	53.00 ± 8.76[b]	20.50 ± 6.14[a]	11.25 ± 13.33[a]	14.50 ± 3.51[a]	25.00 ± 10.80[a]	13.75 ± 10.37[a]

Values are expressed as mean ± SD (n=24)
[a] indicates significant difference (p< 0.05) compared with normal control.
[b] indicates significant difference (p<0.05) compared with negative control

Table IV: Hematological parameters and biochemical analysis of rats after 4 weeks of supplementation with date palm and goat milk

Haematological parameters	Normal control	Negative control	Positive control	Date Palm (DP)	Goat milk (GM)	Date palm + Goat milk (DPGM)
RBC (10^9/L)	8.55 ± 0.34*[,b]	3.65 ± 1.47[a]	8.63 ± 0.53*[,b]	8.25 ± 0.53*[,b]	7.28 ± 1.61*[,b]	8.60 ± 0.08*[, b]
Hb (g/dL)	16.18 ± 0.97*[,b]	6.68 ± 6.18[a]	16.18 ± 0.82*[,b]	15.55 ± 0.62*[,b]	13.85 ± 2.77*[,b]	15.58 ± 0.33*[,b]
PCV (%)	49.75 ± 4.79[b]	16.75 ± 4.50 *[,a]	52.50 ± 3.42*[,b]	49.25 ± 4.86*[,b]	42.75 ± 9.67[b]	50.25 ± 0.96*[,b]
MCV (fL)	58.00 ± 4.69[b]	44.50 ± 4.12 *[,a]	60.75 ± 3.40[b]	60.00 ± 3.16 [b]	59.50 ± 1.7 [b]	58.25 ± 1.71[b]
MCH (pg)	19.00 ± 0.82	15.75 ± 1.26 *	18.75 ± 0.96[b]	19.25 ± 0.50*[,b]	19.25 ± 0.96[b]	18.25 ± 0.50[b]
Serum Iron (µmol/L)	39.90 ± 5.57[b]	3.13 ± 1.49*[,a]	29.35 ± 2.33*[,a,b]	31.95 ± 5.26*[,a,b]	30.48 ± 3.59*[,a,b]	31.95 ± 2.95*[,a,b]
Transferrin saturation (%)	89.25 ± 12.37*[,b]	6.75 ± 2.99*[,a]	49.50 ± 3.70*[,a, b]	61.50 ± 10.97*[,a,b]	76.75 ± 8.42*[,b,c]	60.25 ± 6.24*[,a,b]

Values are expressed as mean ± SD (n=24)
* indicates significant difference (p< 0.05) pre and post treatment
[a] indicates significant difference (p< 0.05) compared with normal control.
[b] indicates significant difference (p<0.05) compared with negative control
[c] indicates significant difference (p<0.05) compared with positive control

by >16% indicating adequate functional iron supply for erythropoiesis. Among the treatment groups, a significant difference (p <0.05) was seen when the goat milk supplemented group was compared with positive control. The goat milk supplemented group also had the highest transferrin level and showed no significant difference with normal control. At the end of the study, serum iron concentration and transferrin saturation in the negative control group were significantly lower (p<0.05) than the normal control group and all treatment groups with value of 3.13 µmol/L and 6.75%, respectively.

DISCUSSION

Phoenix dactylifera and goat milk consumed on a daily basis, are highly beneficial for improving general health and wellbeing. Nutrients present in date palm and goat milk, particularly glucose, fructose, lactose and iron, manganese, calcium, magnesium, zinc and ascorbic acid all contribute to the health and wellbeing of the body. Ajwa dates are rich in sugars particularly glucose and fructose, similar to the previous report by Assirey *et al.* ,(25) with values of 51.3g/100g and 48.5g/100g, respectively. The iron content in Ajwa dates used in this study was particularly high (10.7mg/kg) when compared with that of Hamad *et al.* ,(8) and Khalid *et al.*, (9), each with values of 1.5 and 5mg/kg, respectively. Manganese level measured in Ajwa dates ranged between 3.1 to 5.0mg/kg while zinc content was higher than found in this study (4.6 -12.0mg/kg). The calcium content in Ajwa dates varies; both Hamad *et al.*, (8) and Khalid *et al.*, (9) detected 3.39mg and 20mg calcium respectively, in every kg of Ajwa while Assirey *et al.*, (25) reported 1870mg/kg. Meanwhile, the reported magnesium content of 15mg/kg (9) and 359.4mg/kg (8), are lower than that determined in this study. The variation in nutritional composition in Ajwa dates may probably be due to factors such as harvesting stage of the date palm fruit, genetic variation, soil content and fertilizers used. The maturity stages from Kimri (green colour) to the Tamre (full soft) stage notably affects the mineral composition in Ajwa dates (26).

In this study, goat milk contains 3.38g/100 mL lactose, lower than previously published data in which 4.4g lactose was detected in every 100mL milk (15,27). According to Raynal-Ljutovac *et al.*, (27) and Park *et al.*, (28), the iron concentration in goat milk was 0.05mg/100mL and 0.07mg/100mL, respectively which were slightly lower than iron content detected in this study (0.09mg/100mL). The level of magnesium was higher, with value of 13mg/100 mL and 16mg/100mL, respectively, when compared with this study (10mg/100mL). Raynal-Ljutovac *et al.*, (27) also reported a higher zinc content of 0.34mg/100mL in goat milk, than that determined in this study (0.099mg/100mL).

We determined that the calcium content in goat milk is at 72.2mg/100mL, lower than that reported by Raynal-Ljutovac *et al.*, (27). Factors such as genetic variation, environmental condition and goat farming practice (duration of lactation, milking type, frequency and duration of milking) affect the overall biochemical composition and eventually the quality of milk produced (29).

HRE is used to estimate the percentage of ingested iron that is absorbed and converted into haemoglobin. Recycled iron from macrophage and senescent erythrocytes are typically used for Hb production. However, if the iron supply is inadequate to meet the physiological demands, intestinal iron absorption will be stimulated. During iron deficiency, more iron is being absorbed from the small intestines but when the iron store in the body is high, the iron absorption in small intestine decreases. The efficiency of haemoglobin recovery in anaemic subjects reflects the dietary iron incorporation into haemoglobin over the course of treatment period. Numerous factors in the diet may influence mineral bioavailability in the small intestine, which depends on presence or absence of enhancer and inhibitor in the food and hence the food matrix (30).

In iron deprivation state, haematological parameters in the IDA subjects drastically differ from those of normal control with reduced RBC, Hb concentration, PCV, MCV and MCH level. Iron profile with reduced serum iron and transferrin saturation is consistent with the iron deficiency state. Serum iron concentration and transferrin saturation are frequently used as indicators for the status of iron in the body (23). In iron deficiency, the iron available for erythropoiesis is restricted, leading to reduced serum iron concentration and transferrin saturation. Reference range for transferrin saturation is between 20% - 50% and low transferrin saturation of 18% and below indicate an inadequate iron supply to support new haemoglobin synthesis and red blood cell production (31).

In this study, supplementation of date palm and goat milk in IDA rats had significantly promoted the production of RBC after 4 weeks of treatment, showing complete recovery from iron deficient state. Improvement in Hb concentration, PCV, MCV and MCH were also observed. These findings are in line with the previous report by Onuh *et al.*, (14) who showed an improvement in haemopoietic activity when crude methanolic and crude aqueous extract of date palm fruit were used in anaemic rats. Abdelsalam *et al.*, (13) showed a significant increase in Hb concentration in anaemic late pregnant Najdi ewes when treated with sukkary date palm extracts. Zen *et al.*, (32) demonstrated that date palm juice significantly increased the haemoglobin level in male rats fed on low iron diet. These finding suggest that date palm, regardless of the variety and type of extraction is rich in beneficial nutritional contents, that supports haemoglobin and erythropoietic synthesis. Goat milk consumption was reported to improve iron metabolism, favouring iron deficiency anaemia recovery. Diaz-Castro *et al.*, (33) revealed that goat milk improved haematological parameters, restored liver iron content and significantly increased the iron carrier protein, DMT1 expression in anaemic rat. Among the supplemented groups, rats fed on goat milk singly, showed lower Hb value compared with the date palm and combination supplemented groups. This may be probably due to the low iron content in the goat milk used.

Greater utilisation of iron with date palm and goat milk diet could be due to numerous nutritional factors. Date palm is rich in carbohydrate, dietary fibre, vitamin and minerals and can be considered as an ideal food which provides a wide range of vital nutrients with numerous health benefits. Date palm contains a high percentage of carbohydrate in the form of reducing sugars, namely fructose and glucose that are readily absorb during digestion, providing instant energy. Dietary fibre induces satiety and has laxative effect thus can prevent spectrum of gastrointestinal disorder (34). Date palm also contains minerals such as zinc, magnesium, iron and calcium that are essential for optimum growth and maintenance of human body. Date palm is rich in ascorbic acid, which enhanced iron uptake by reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). Ascorbic acid was also reported to chelate iron and stimulates the synthesis of ferritin (11). Besides, date palm fruit also rich in phytochemicals such as carotenoids, phytosterols, phenolic acids, flavonoids, tannins and sterol which may give health benefit when taken as a medicine drug or as a part of daily diet (35). Identification and quantification of different classes of phytochemicals as well as pharmacological studies (in vitro and in vivo) showed therapeutic effects of date palm on health as their consumption has been linked to anti-inflammatory activity (36), anti-cancer activity (37), antifungal activity (38) anti-bacterial activity (39) and protective activity against toxicity (40).

Goat milk on the other hand, contains a greater proportion of soluble proteins such as β-lactoglobulin, α-lactoalbumin and serum albumin which favour iron absorption in small intestines (41). Goat milk also contains a high amount of medium chain triglycerides (MCT), a form of saturated fatty acid with 6-10 carbon that are readily absorbed within the intestinal cells, contributing to easier and efficient digestion. MCT is rapidly hydrolysed and metabolised for energy discharge with lower deposit of fat. It escalates the synthesis of protein carriers that lead to increased nutrient absorption (16). Higher level of iron digestibility was reported in rats with distal small intestines resection when supplemented with goat milk. Greater iron content was observed in the liver, spleen and sternum, suggesting that short-chain fatty acids favour intestinal adsorption after resection (42). Nestares *et al.*, (43) reported that

despite the high calcium content, goat milk diet resulted in minimal calcium-iron interaction and did not negatively affect the iron status in IDA rats. In addition, various dietary components in goat milk play vital roles in iron utilisation. High content of Vitamin D in goat milk promotes erythropoiesis by increasing erythroid progenitor proliferation (44) while vitamin A influences iron metabolism by mobilizing available iron from the ferritin store to form haemoglobin (45). The positive effect of goat milk on iron absorption and bioavailability have been studied in anaemic subjects particularly in animal (46–48). A recent systematic review also reported the beneficial effect of goat milk in iron deficiency anaemia (49). Numerous studies showed the beneficial effects of date palm and goat milk on hemopoiesis in anaemic subjects. This study demonstrated that date palm and goat milk singly or in combination have good iron bioavailability and are good natural sources to replenish iron.

CONCLUSION

This study demonstrated that supplementation of date palm and goat milk favours the recovery from iron deficiency anaemia in rat. It improves the haematological parameters as well as the bioavailability of functional iron for erythropoiesis. Supplementation of date palm and goat milk on a daily basis is effective in alleviating iron deficiency anaemia in rat.

ACKNOWLEDGEMENTS

The authors would like to thank Universiti Sains Islam Malaysia and The Ministry of Higher Education Malaysia (MOHE) for financial support through Niche Research Grant Scheme (USIM/NRGS_P9/ FPSK/8409/52113).

REFERENCES

1. WHO. The Global Prevalence of Anaemia in 2011. Geneva: World Health Organisation 2015; 2015. 1–19 p.
2. Camaschella C. Iron-Deficiency Anemia. N Engl J Med. 2015;372(19):1832–43.
3. Heaney R. Bioavailability of Nutrients and Other Bioactive Components from Dietary Supplements. J Nutr. 2001;131(4):1376–82.
4. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr. 2010;91(5):1461S-1467S.
5. Hunt J. Moving toward a plant-based diet: Are iron and zinc at risk? Nutr Rev. 2002;60(5 I):127–34.
6. Eid NMS, Al-Awadi B, Vauzour D, Oruna-Concha MJ, Spencer JPE. Effect of cultivar type and ripening on the polyphenol content of date palm fruit. J Agric Food Chem. 2013;61(10):2453–60.
7. Khalid S, Khalid N, Khan R., Ahmed H, Ahmad A. A review on chemistry and pharmacology of Ajwa date fruit and pit. Trends Food Sci Technol. 2017;63:60–9.
8. Hamad I, Abdelgawad H, Al Jaouni S, Zinta G, Asard H, Hassan S, *et al*. Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) cultivars from Saudi Arabia to assess their nutritional quality. Molecules. 2015;20(8):13620–41.
9. Khalid S, Ahmad A, Masud T, Asad MJ, Sandhu M. Nutritional assessment of ajwa date flesh and pits in comparison to local varieties. J Anim Plant Sci. 2016;26(4):1072–80.
10. Al-Shahib W, Marshall RJ. The fruit of the date palm: Its possible use as the best food for the future? Int J Food Sci Nutr. 2003;54(4):247–59.
11. Lane DJR, Richardson DR. The active role of vitamin C in mammalian iron metabolism: Much more than just enhanced iron absorption! Free Radic Biol Med. 2014;75:69–83.
12. Heffernan A, Evans C, Holmes M, Moore JB. The Regulation of Dietary Iron Bioavailability by Vitamin C: A Systematic Review and Meta-Analysis. Proc Nutr Soc. 2017;76(OCE4):4433.
13. Abdelsalam MM, Zeitoun MM, Ateah MA, Al-Hassan A, Abdel-Salam AM. Impact of probiotic fermented milk, palm date extract and their mixture supplementation on neonatal traits and hematological parameters of late pregnant najdi ewes. Vol. 8, International Journal of Biological Chemistry. 2014. p. 37–47.
14. Onuh SN, Ukaejiofo EO, Achukwu P., Ufelle SA, Okwuosa CN, Chukwuka CJ. Haemopoietic activity and effect of Crude Fruit Extract of *Phoenix dactylifera* on Peripheral Blood Parameters. Int J Biol Med Res. 2012;3(1):1720–3.
15. Turkmen N. The Nutritional Value and Health Benefits of Goat Milk Components. In: Ronald W, Collier R., Preedy V, editors. Nutrients in Dairy and Their Implications for Health and Disease. Elsevier Inc.; 2017. p. 441–9.
16. Dнaz-Castro J, Lisbona F, Moreno M, Alfйrez M, Campos M, Lypez-Aliaga I. Influence of Goat Milk on Iron Deficiency Anemia Recovery. Int J Dairy Sci Process. 2015;2(1):7–11.
17. Lynch SR. The effect of calcium on iron absorption. Nutr Res Rev. 2000;13(2):141–58.
18. Alfйrez MJM, Lypez-Aliaga I, Nestares T, Dнaz-Castro J, Barrionuevo M, Ros PB, *et al*. Dietary goat milk improves iron bioavailability in rats with induced ferropenic anaemia in comparison with cow milk. Int Dairy J. 2006;16(7):813–21.
19. Hodgkinson AJ, McDonald NA, Kivits LJ, Hurford DR, Fahey S, Prosser C. Allergic responses induced by goat milk αS1-casein in a murine model of gastrointestinal atopy. J Dairy Sci. 2012;95(1):83–90.
20. Da Costa WKA, De Souza EL, Beltrao-Filho EM, Vasconcelos GKV, Santi-Gadelha T, Gadelha CADA, *et al*. Comparative protein composition analysis of goat milk produced by the alpine and

saanen breeds in Northeastern Brazil and related antibacterial activities. PLoS One. 2014;9(3).
21. Al-Qarawi AA, Abdel-Rahman H, Ali BH, Mousa HM, El-Mougy SA. The ameliorative effect of dates (*Phoenix dactylifera* L.) on ethanol-induced gastric ulcer in rats. J Ethnopharmacol. 2005;98(3):313–7.
22. Jacob Filho W, Lima CC, Paunksnis MRR, Silva AA, Perilhгo MS, Caldeira M, *et al*. Reference database of hematological parameters for growing and aging rats. Aging Male. 2017;21(2):145–8.
23. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J basic Clin Pharm. 2016;7(2):27–31.
24. Mahoney, VanOrden., Hendricks. Efficiency of Converting Food Iron into Hemoglobin by the Anemic R at1. 1974;230(1820):223–30.
25. Assirey EAR. Nutritional composition of fruit of 10 date palm (*Phoenix dactylifera* L.) cultivars grown in Saudi Arabia. J Taibah Univ Sci. 2015;9(1):75–9.
26. Iqbal M, Munir M, Niamatullah M. Physio-Chemical Characteristics of Date Palm (*Phoenix Dactylifera* L .) Cultivars At Various Maturity Stages Under Environmental. J Agrriculture Res. 2011;49(6):249–61.
27. Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I, Chilliard Y. Composition of goat and sheep milk products: An update. Small Rumin Res. 2008;79(1):57–72.
28. Park Y, Juбrez M, Ramos M, Haenlein G. Physico-chemical characteristics of goat and sheep milk. Small Rumin Res. 2007;68(1–2):88–113.
29. Yangilar F. As a Potentially Functional Food: Goats' Milk and Products. J Food Nutr Res. 2013;1(4):68–81.
30. Gibson RS. The role of diet- and host-related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates. Food Nutr Bull. 2007;28(1 SUPPL. 1):77–100.
31. Auerbach M, Adamson JW. How we diagnose and treat iron deficiency anemia. Am J Hematol. 2016;91(1):31–8.
32. Zen ATH, Pertiwi D, 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. 2013;5(1):17–9.
33. Díaz-Castro J, Pulido M, Alfйrez MJM, Ochoa JJ, Rivas E, Hijano S, *et al*. Goat milk consumption modulates liver divalent metal transporter 1 (DMT1) expression and serum hepcidin during Fe repletion in Fe-deficiency anemia. J Dairy Sci. 2014;97(1):147–54.
34. Mrabet A, Hammadi H, Rodrнguez-Gutiйrrez G, Jiménez-Araujo A, Sindic M. Date palm fruits as a potential source of functional dietary fiber: A review. Food Sci Technol Res. 2019;25(1):1–10.
35. Al-Alawi R, Al-Mashiqri JH, Al-Nadabi JSM, Al-Shihi BI, Baqi Y. Date palm tree (*Phoenix dactylifera* L.): Natural products and therapeutic options. Front Plant Sci. 2017;8(MAY):1–12.
36. Zhang C-R, Aldosari SA, Vidyasagar P, Nair K, Nair M. Antioxidant and Anti-in fl ammatory Assays Con fi rm Bioactive Compounds in Ajwa Date Fruit. J Agric Food Chem. 2013;61(24):5834–40.
37. Siddiqui S, Ahmad R, Khan MA, Upadhyay S, Husain I, Srivastava AN. Cytostatic and Anti-tumor Potential of Ajwa Date Pulp against Human Hepatocellular Carcinoma HepG2 Cells. Sci Rep. 2019;9(1):1–12.
38. Belmir S, Boucherit K, Boucherit-Otmani Z, Belhachemi MH. Effect of aqueous extract of date palm fruit (*Phoenix dactylifera* L.) on therapeutic index of amphotericin B. Phytotherapie. 2016;14(2):97–101.
39. Samad MA, Hashim SH, Simarani K, Yaacob JS. Antibacterial Properties and Effects of Fruit Chilling and Extract Storage on Antioxidant Activity, Total Phenolic and Anthocyanin Content of Four Date Palm (*Phoenix dactylifera*) Cultivars. Molecules. 2016;21(4):1–14.
40. Bashandy MA, Abd-el-aal A, Ibrahim DF, El-sharkawy MA. Protective effects of date palm extract as natural antioxidants on hepatotoxicity induced by Cerastes cerastes venom in albino rats. Int J Adv Res. 2016;4(3):647–65.
41. Lypez-Aliaga I, Dнaz-Castro J, Alfйrez MJM, Barrionuevo M, Campos MS. A review of the nutritional and health aspects of goat milk in cases of intestinal resection. Dairy Sci Technol. 2010;90(6):611–22.
42. Barrionuevo M, Alferez MJM, Lopez Aliaga I, Sanz Sampelayo MR, Campos MS. Beneficial Effect of Goat Milk on Nutritive Utilization of Iron and Copper in Malabsorption Syndrome. J Dairy Sci. 2002;85(3):657–64.
43. Nestares T, Diaz-Castro J, Alferez MJ, Lopez-Aliaga I, Barrionuevo M, Campos MS. Calcium-enriched goat milk, in comparison with similarly enriched cow milk, favours magnesium bioavailability in rats with nutritional ferropenic anaemia. J Sci Food Agric. 2008;88:319–27.
44. Smith EM, Tangpricha V. Vitamin D and anemia: Insights into an emerging association. Curr Opin Endocrinol Diabetes Obes. 2015;22(6):432–8.
45. Michelazzo FB, Oliveira JM, Stefanello J, Luzia LA, Rondy PHC. The influence of vitamin A supplementation on iron status. Nutrients. 2013;5(11):4399–413.
46. Dнaz-Castro J, Alfйrez MJM, Lypez-Aliaga I, Nestares T, Campos MS. Effect of calcium-fortified milk-rich diets (either goat's or cow's milk) on copper bioavailability in iron-deficient anemia. Dairy Sci Technol. 2011;91(2):203–12.
47. Park YW, Mahoney AW, Hendricks DG. Bioavailability of iron in goat milk compared with cow milk fed to anemic rats. J Dairy Sci. 1986;69(10):2608–15.

48. Alférez MJM, Lypez-Aliaga I, Nestares T, Dнaz-Castro J, Barrionuevo M, Ros PB, *et al*. Dietary goat milk improves iron bioavailability in rats with induced ferropenic anaemia in comparison with cow milk. Int Dairy J. 2006;16(7):813–21.

49. Zahir NNM, Zulkifli NF, Hamid NA, Shamaan NA, Asnawi AWA, Rahim NS, *et al*. A systematic review on the beneficial effects of goat milk on iron deficiency anaemia. Vol. 23, Advanced Science Letters. 2017. p. 23 (5), 4824–30.