



Original Article

Evaluation of the anti-anemic activity of date syrup in Wistar rats LAICHE Ammar Touhami ^{a,b*}, GHEMAM HAMED Amina ^a, BADI Salima ^a

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ABSTRACT

The main objective of this study is to determine the effect of food intake of date syrup on the treatment of anemia.

First, we used 3 samples of dates from different regions for the preparation of the syrup. We have studied certain criteria (physical, chemical, biochemical, microbiological); where the results showed that the prepared date syrup has a good hygienic quality, and of remarkable nutritional quality due to its ideal content with many properties, such as carbohydrate and protein content.

To evaluate the anti-anemic properties of date syrup in rats, anemia is induced by a food having an iron deficiency. Date syrup, prepared from Djamaa dates, was administered by gavage of anemic rats at doses of 1000 mg / kg / day and 2000 mg / kg / day resulting in an increase in hemoglobin, red blood cell count, hematocrit and serum iron. Thanks to the results obtained, date syrup can be classified among the foods that help fight iron deficiency anemia.

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1. Introduction

World production of dates is estimated at 7,416,000 tons. Algeria is one of the major date producers, occupying the fifth class in the world, the number of these trees is estimated at 13 million palms and 940 cultivars, with a total production estimated at 848,199 tonnes [Error! Reference source not found.]. Dates are the subject of significant commercial activity, especially the Deglet-Nour variety; it has a monopoly on national and international markets [2].

Indeed, the high content of sugar and nutrients, justify their use as raw materials in the manufacture of various food products with high added value such as date juice, jam, date syrup, date paste, date flour ... [3].

Date syrup is a product of high nutritional value; it is rich in carbohydrates, minerals, B vitamins, phenolic compounds and medium content of flavonoids. These antioxidants reduce the risk of degenerative diseases and

certain types of cancer by reducing oxidative stress and inhibiting the oxidation of macromolecules. Given its richness in mineral salts, especially calcium and iron, date syrup can play an important role in the treatment of anemia [4].

Anemia is defined according to WHO as a pathological condition in which the hemoglobin content in the blood has become abnormally low following a deficiency of one or more essential nutrients. Anemia can be easily treated with a healthy diet. Although there are other types of anemia, which are serious and can also pose a threat to an individual's life [5].

Few studies have exploited the therapeutic effect of date syrup against different types of anemia, The present work aims to enhance a date by-product, with low market value, by manufacturing a syrup of biochemical and microbiological quality in accordance with standards; thus,

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use this syrup in the treatment of deficiency anemia.

2. Materials and Methods

2.1. Plant material, Collection and storage of samples

The plant material used in our study was the fruit of the date palm (*Phoenix dactylifera L*) of the variety “El Ghars”. The dates were taken at the stage of complete maturation (Tamer stage). The choice of this variety is justified by its relative abundance on the national territory, taste quality and its wide consumption throughout the Algerian territory as well as its traditional therapeutic virtues, in particular represented by its richness in minerals.

The dates used in this work were harvested in October 2019 from three different palm groves in the Daïra of “Hassi Khalifa, El Oued and Djamaa” in El-Oued city; Algeria. In order to preserve the dates, we sorted the infested dates before packaging them in a plastic container until the air was expelled and then covered tightly; it is stored at room temperature. In this form, dates can be kept for three years [6].

2.2. Date syrup preparation

To have a good quality product, we have to start from a good quality raw material, so we started by sorting, washing and drying all the dates. We have carried out the following steps:

- **Sorting:** To eliminate of all immature dates, crushed dates or dates attacked by birds and insects. The sorting of dates was carried out entirely by hand [7].
- **Washing:** To remove soil particles, grains of sand, dust, plant debris, treatment products and pests. It was done with tap water. This is an essential step to have a product of good hygienic quality [7].
- **Soaking:** The dates were then subjected to drying by draining through a colander, followed by exposure to air and to room temperatures to remove excess water for one day [7].
- **Pitting and cutting:** To remove pits and done by hand in order not to interfere with the crushing process and to avoid damage by the pits. Dates were cut into small pieces to increase the contact surface with the water and to extract as much juice as possible [7].
- **Juice extraction from dates:** The extraction was carried out hot at a temperature of 85 °C by the addition of 3 volumes of water (pH = 7.2) was used in one volume of date, mixing for half -hour after the mixture reaches the extraction temperature to facilitate the solubility of sugars in water by crushing the date pulp [4,8].
- **Concentration of date juice:** The concentration of the juice was carried out by direct heating at a temperature varying between 100 and 105 °C to remove free water, for 2 hours [9].

2.3. Characterization of the hygienic quality of date syrups

The product obtained after extraction must undergo the following physicochemical, biochemical and microbiological analyzes:

- ✓ **Physicochemical parameters:** determination of pH, electrical conductivity, titratable acidity, soluble solids level, humidity level, ash content.
- ✓ **Biochemical parameters:** Determination of the total sugars content according to [10], of proteins according to [11].
- ✓ **Microbiological parameters:** in order to ensure that the prepared product has a hygienic quality which characterizes the risk to the health of the consumer and the commercial quality; namely the search and enumeration of: total mesophilic flora (Nutrient agar) at 37 °C for 24 and 48 hours; total coliforms (VRBG agar) at 37°C for 24 hours; enterobacteriaceae (Hektoen agar) at 37 °C for 24 and 48 hours; staphylococcus (Chapman agar) at 37 °C for 24 and 48 hours and yeasts and molds (Sabouraud agar) at 37 °C for 05 days.

2.4. In vivo study of the antianemia activity of date syrup

20 rats (adult male) were used in this experiment, their average body weight was 150 to ± 25g. During the period of this experiment, the rats were kept at a temperature of 25 °C and a natural photoperiod, fed a standard well-balanced diet and drink tap water. They were treated in accordance with the principle set out in the manual on the care and use of experimental animals.

2.4.1. Induction of anemia in rats

After adaptation for 15 days, the rats were randomly assigned to the control group and the model groups with iron deficiency anemia. Five rats were randomly selected and given a normal diet as a control group. Others were given a low iron diet (coarse corn powder with an average iron content of 12mg / kg) for 4 weeks, with bleeding undertaken by puncturing the lateral tail veins three times a week (1, 0 - 1.5 ml of blood each time), to generate an animal model with iron deficiency anemia. While the rats in the control and model groups received deionized distilled water [12]. The rats were divided into 4 groups of 5 rats each, these are:

- **Lot 01:** non-anemic control;
- **Lot 02:** untreated anemic rats;
- **Lot 03:** anemic rats treated with date syrup at a dose of 1000 mg / kg of body mass per day by gavage, for 2 weeks;
- **Lot 04:** anemic rats treated with date syrup at a dose of 2000 mg / kg of body mass per day by gavage, for 2 weeks.

2.4.2. Blood sampling and determination of anemic parameters

At the end of the experimental protocol, the rats were fasted for 12 h before blood collection. Blood samples

were taken by puncture of the lateral tail veins and blood was collected in EDTA tubes and dry tubes, for the purpose of performing laboratory-level bioassays.

The haematological analysis was carried out directly (after one hour of sampling) in order to avoid the autolysis of the cells and to obtain reliable results. The hematological analysis was determined on an automatic counter model BC 2800. This device is intended for the automatic haematological analysis which gives information on white blood cells, red blood cells, platelets, hematocrit (HCT), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin content (TCMH) [13].

3. Results and Discussion

3.1. Characterization of the hygienic quality of date syrups

3.1.1. Physicochemical and biochemical parameters of date syrup

The results of the physicochemical and biochemical analysis are presented in the following table

Table 01: Physicochemical and biochemical parameters of date syrup

Our results indicate that the physicochemical and

Parameters	Date syrup		
	Hassi Khalifa average \pm SD	El Oued average \pm SD	Djamaa average \pm SD
pH	5.06 \pm 0.10	4.99 \pm 0.11	4.99 \pm 0.19
E.C μ S/cm	4.63 \pm 0.50	3.81 \pm 0.48	5.53 \pm 0.58
titrable acidity g/100g	0.64 \pm 0.07	0.59 \pm 0.48	0.68 \pm 0.04
soluble solids level ⁰ Brix	59.75 \pm 0.12	60 \pm 0.50	68 \pm 0.0
humidity %	44.22 \pm 0.2	42.8 \pm 0.27	36.36 \pm 0.25
Dry matter%	55.78 \pm 0.27	57.18 \pm 0.27	34.07 \pm 0.25
ash content %	5.3 \pm 0.36	5.3 \pm 0.36	10.2 \pm 0.24
Proteins %	3.40 \pm 0.08	2.30 \pm 0.080	7.30 \pm 0.06
Total sugar %	49 \pm 0.32	42 \pm 0.128	27 \pm 0.12

biochemical quality of different samples used are almost identical, these values relate to the variety (*Ghars*) from different sampling regions.

The analysis of the results obtained indicates that the measured pH values show a slight difference between the three samples of date syrups. These results are lower compared to those obtained by [14], for the Ghars variety (pH 5.64), and higher compared to those obtained by [15]. The highest electrical conductivity content of date syrup is observed at the level of Djamaa date syrup at $5.53 \pm 0.58 \mu\text{S} / \text{cm}$. These results are comparable to those obtained by [9], for date syrup from the Ghars variety after an extraction with concentration by direct heating which show values of $5.51 \mu\text{S} / \text{cm}$.

The lowest water contents were $36.36 \pm 0.25\%$ of the Djamaa syrup, followed by the syrup of El Oued ($42.82 \pm 0.27\%$) and Hassi Khalifa ($44.22 \pm 0.20\%$). Our results are different with those found by [16,3]. The variations in

humidity levels are probably due to extraction methods, climatic conditions, storage and the type of date varieties used [17].

The syrup dry matter is inversely related to the water content. These results showed the lowest content of DM are observed in Djamaa date syrups ($34.07 \pm 0.25\%$), compared to that of Hassi Khalifa date syrup ($55.78 \pm 0.27\%$) and date syrup of El Oued ($57.18 \pm 0.27\%$). Our dry matter content values are lower than those of [3] (84%) and [15] (77.88%).

The ash content of our samples is respectively $10.2 \pm 0.2\%$, $5.3 \pm 0.36\%$ and $4.5 \pm 0.15\%$ for the syrup from Djamaa, El Oued, Hassi Khalifa. Our results are comparable to those recorded by [3] (6.8%). Likewise, they are higher than those mentioned by [15] by 0.96% for the Ghars variety.

The results obtained concerning the protein level seem higher compared to those advanced by [3, 18, 19]. According to these authors, date syrup contains 0.83%, 2.2%, 1.09% of protein, respectively.

The means of the total sugars contents of the three samples show that the date syrup from Hassi Khalifa has the highest content ($49 \pm 0.0325\%$). The values are lower than the value found by [16] (74%) and [20] (73%), [3] (79.45%) and [21] (80%).

These differences in biochemical parameters may be due to various factors such as the type of variety, the growing conditions of the variety, its stage of maturity, its geographical origin, the type of soil and the storage conditions of dates [22].

So from our results about physicochemical and biochemical quality, we can say that our samples of date syrup from different regions contain a considerable amount of the essential elements which qualifies it as good quality and conforms to the standards and previous studies.

3.1.2. Microbiological parameters of date syrup

The results of the microbiological analysis of different samples of date syrup are summarized in the following figure. The colonies appearing in the Nutrient Agar medium give us an idea of the total flora contained in our samples. Date syrup from Djamaa dates represents the highest load ($2.4 \text{ Log CFU} / \text{ml}$) compared to those from the region of El Oued and Hassi Khalifa. According to [23], the total mesophilic aerobic flora can contain the germs: *Staphylococcus*, *Streptococcus*, *Lactococcus*, *Corynebacterium*, *Bacillus*, *Pseudomonas* and *Acromobacter*.

The analysis of the results obtained shows that the number of Enterobacteriaceae, cultivated in Hectoen medium, in date syrup from Hassi Khalifa, El Oued and Djamaa are respectively $1.54 \text{ log UFC} / \text{ml}$, $0.91 \text{ log UFC} / \text{ml}$ and $1.56 \text{ log UFC} / \text{ml}$, which are lower than the standards recommended for dry products by the order of July 2017 relating to the microbiological specifications of certain foodstuffs: international standards ($<103 \text{ CFU} / \text{ml}$).

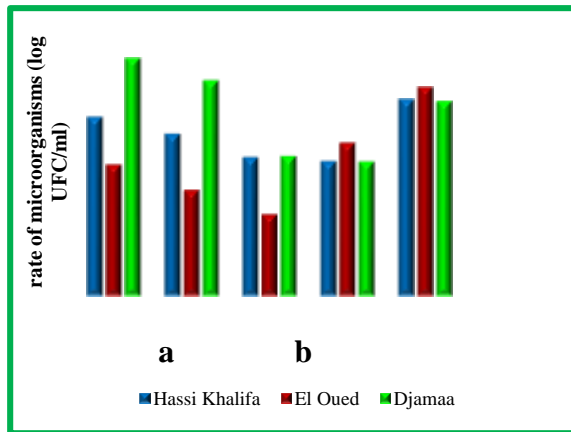


Figure 01: Enumeration of different groups in date syrups
 a. Total mesophilic flora; b. Enterobacteriaceae; c. *Staphylococcus*; d. Yeasts and molds; e. Total coliforms

The results obtained from the research of *Staphylococcus aureus*, she specified that it is slightly present in El Oued syrup (0.91 log CFU / ml) compared to that of Hassi Khalifa syrup (0.54 log CFU / ml) and that of Djamaa (0.56 log CFU / ml). However, are lower than the international standards recommended for dry products (<103 CFU / ml). The other species of staphylococci generally produce smaller colonies, not causing the color change [24].

For total coliform bacteria, the analysis of the results shows that El Oued syrup contains 1.7 log CFU / ml and date syrup from Hassi Khalifa and Djamaa are 1.5 log CFU / ml, 1 which are lower than international standards (< 103 CFU / ml according to international standards).

The results of Yeasts and molds show 2.32 log CFU / ml (<104 CFU / ml according to the international standards recommended for dry products) in the sample of El OUED syrup, and 2.19 log CFU / ml for Hassi Khalifa syrup and 2.32 log CFU / ml in the sample from Djamaa.

The fermentation of lactose into lactic acid, due to the presence of coliforms in the VRBG medium, is revealed by a change in the color of the colonies to pink and brown caused by the precipitation of bile salts [24].

From the results obtained, it can be seen that the date syrup is of good hygienic quality. Our results are comparable to standards for concentrated products (3.103 for TMF and <103 for yeasts and molds).

These results can be explained by the physicochemical properties of date syrup rich in antioxidants (phenolic content is around mg / 100g Ms) which are responsible for the inhibition of the proliferation of these germs and by the pH which constitutes one of the main obstacles that the microbial flora must overcome to ensure its proliferation [9].

3.2. *In vivo* study of the antianemia activity of date syrup

The results relating to the determination of the haematological parameters (linked to anemia) of the rats treated in the present study are shown in the following figures (02 and 03).

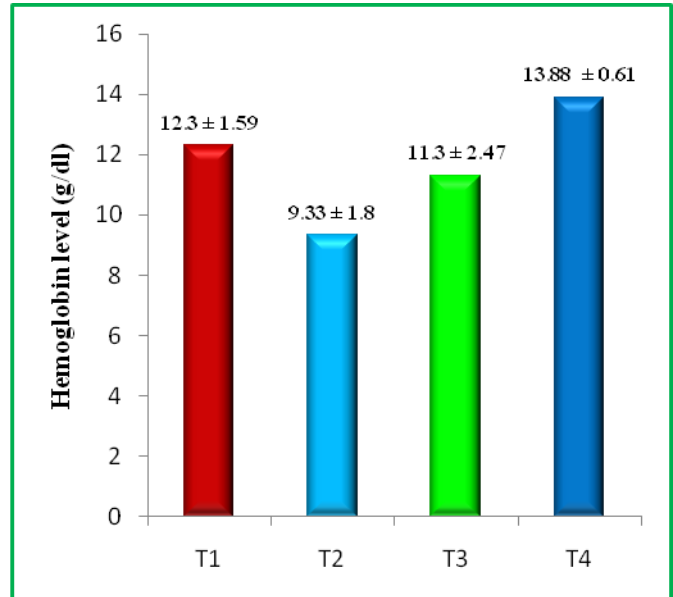


Figure 02: Hemoglobin level (g / dl) and Percentage of hematocrit (%) in the blood of various experienced rats
 White control rats (T1); Negative control rats (T2);
 Rats having received 1000 (T3) mg / Kg of date syrup,
 Rats having received 2000 mg / Kg of date syrup (T4).

Red blood cell, hemoglobin, and hematocrit levels were lower in anemic rats than in normal mice. The level of red blood cells, hemoglobin, and hematocrit increased significantly after consuming date syrup. The level of haematological parameters in the high dose group was significantly higher than in the 1000 mg / Kg / d group, which indicates that the high dose 2000 mg / Kg / d was more effective in relieving the symptoms of anemia. Our results are close to those of [12]; who observed a decrease in the number of hemoglobin and hematocrit with the administration of an iron deficient diet and bleeding for 4 weeks in rats.

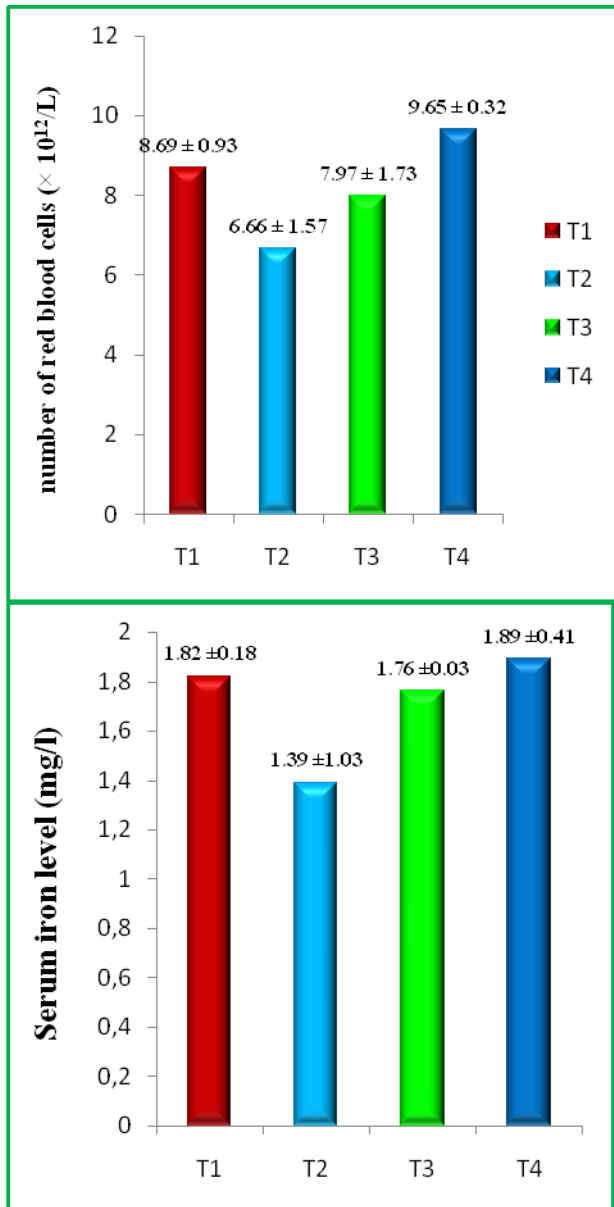


Figure 03: Number of red blood cells ($\times 10^{12} / L$) and Serum iron concentration in the blood of various experienced

White control rats (T1); Negative control rats (T2); Rats having received 1000 (T3) mg / Kg of date syrup, Rats having received 2000 mg / Kg of date syrup (T4).

Measurement of serum iron levels is useful for the clinical diagnosis of iron deficiency anemia. Serum iron is the amount of rotating iron that needs to be transported. Our observation showed that iron deficiency anemia significantly reduced serum iron levels in rats. On the other hand, treatment with date syrup may increase the iron level in the blood of iron deficient rats. The serum iron level of the rats in the high dose group (2000 mg / Kg / d) showed significant difference compared to the control group but was much higher than that of the 1000 mg / Kg / d group. The results indicated that date syrup at a high dose has a significant effect on improving iron deficiency anemia.

[25, 12] also obtained similar results in reduction of serum iron level with administration of iron deficient diet and bleeding for 4 weeks in rats. Iron is necessary for the synthesis of hemoglobin, so it is reasonable to assume that any iron deficiency will result in a slower rate of hemoglobin synthesis. The results showed a marked decrease in hemoglobin and serum iron in mice fed an iron-deficient diet [12].

The results show that date syrup is of great importance in the therapeutic effect of iron deficiency anemia. The first reason perhaps lies in the fact that this drink has a high bioavailability of iron "and that the presence of proteins, carbohydrates and fats and elements such as Zn, Fe and Ca and the presence of abundant amounts of vitamin A contributes to the synthesis of hemoglobin. Dietary modification may play a central role in the treatment of anemia [26].

Patients with nutritional anemia due to iron deficiency should be educated about foods rich in iron. Foods like green leafy vegetables, tofu, red meats, raisins, and dates contain a lot of iron [27].

Dates exhibit anti-cancer properties and are also a good source of natural antioxidants. Dates can improve hemoglobin (Hgb) levels by increasing erythrocyte production [28]. [29] found that the use of dates in the treatment of deficiency anemia in children is beneficial and the iron content.

[30] found that date extract increases the level of Hgb, which indicates that dates are rich in iron and the presence of proteins, carbohydrates, fats and elements such as Zn, Fe, Ca and the presence of abundant amounts of vitamin A helps in the synthesis of Hgb.

This suggests that nutritional correction plays a central role in controlling anemia. Considering the many properties of dates and its various ingredients, it is suggested that this cheap, healthy and affordable fruit should be considered in the primary school feeding program [28].

Conclusion

The syrups produced in our study have a good hygienic quality. Their nutritional quality is also appreciable since their low carbohydrate content is between 27 - 49%; protein is between 2.30 and 7.30%. In addition, our samples are of microbiological quality in accordance with standards and their consumption does not present any contamination problem.

Administration of date syrup by gavage at doses of 1000 mg / kg / day and 2000 mg / kg / day significantly increased the hemoglobin level and the number of red blood cells, hematocrit of serum iron per day 16. The dose of 2000 mg / kg / day allowed complete recovery of the hematological parameter and the serum iron level of the rats on day 16 compared to the control rats. Date syrup is of great importance in the therapeutic effect of iron deficiency anemia, due to its richness in iron and other properties contribute to the synthesis of hemoglobin.

Our results confirm and validate the traditional therapeutic indication of date syrup in the absorption of anemia. This

syrup could, according to our experimental conditions, therefore be recommended to reduce anemia complications.

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ensuring the breeding and treatment of rats, as well as carrying out the necessary analysis.

Conflict of Interest

The authors declare that they have no conflict of interest in this work

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