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Original Article

Investigation of Hematological and Biochemical Effects of Feeding Date in the Early Morning on Empty Stomach vs. after Nutrition on Rabbits

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Abstract

Date seeds have been studied for their possible health advantages as they are employed in various traditional remedies. This study aimed to investigate how date affected hematology, renal, and liver function in rabbits before and after date feeding. In total, 30 rabbits were used in this investigation, and they were divided into two groups (n=15). Group one (G1) was considered the control group and received only a meal without dates for 30 days, and group two (G2) was given date seed extract a about 30 ml/kg b.w. for 30 days. The findings revealed that daily oral administration of date extract resulted in a considerable increase in hemoglobin (Hgb) concentration. It is now recognized as a useful source of natural therapeutic ingredients for a variety of ailments. The study results showed that the oral administration of dates led to a significant increase in Hgb concentration, Hgb indices (MCH, MCV, MCHC, PLT, WBCs, and RBCs) and a significant increase in total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine, and blood urea nitrogen levels ($P \leq 0.05$). However, there were no significant changes in albumin in G2, compared to G1. Finally, dates may help to increase biochemical and hematological parameters in rabbits.

Keywords: Biochemical Parameters, Blood Parameters, Dates, Physiological Activities, Rabbits

1. Introduction

Dates for centuries have been utilized as food being staple and no less than 800 utilizations are verified for it (1). The family of palm is considered the Muslims' love and prosperity symbol, and its folklore is traced back to the mythology of Judeo-Christian. In native medicinal uses, dates are considered tonic nutrients, and others regard dates as an aphrodisiac. On the other hand, the date flowers are used as a digestive tract purgative (2). Results of the previously published study showed that the extracts of date significantly increase sperm count and also lead to a significant improvement in spermatogenesis in guinea pigs.

On the other hand, in rats, the date extract (DE) elevates the testosterone concentration, follicle-stimulating hormone, and luteinizing hormone (1). The date pollen grains have been utilized in Iraq for improving women's fertility (3). Pits of date have been mixed in the feed of rats as the growth enhancer, an action that was attributed to the elevation in the plasma levels of testosterone or estrogens (4). To the best of the authors' knowledge, there have not been any studies in the animal models to investigate the effect of DE on cardiovascular disease, renal function, stress, or antioxidants capacity (5). Furthermore, there has not been any research to investigate the effects of date

fruits on total complete blood count, lipid peroxidation, and serum biochemical parameters in rabbits. In the Middle East, it is widely accepted that dates consumption, mostly on an empty stomach in the early morning can exert anti-cytotoxic effects (6).

The effect of date (*Phoenix dactylifera*; Arecacaea) extracts on *Streptococcus pyogenes* was examined *in vivo* and *in vitro*. It was found that the incubation for 24 h with date fruit extract at 5%, 10%, and 20% dilution effectively slowed the growth of *S. pyogenes* to 30.8%, 64.7%, and 88.5%, respectively. DE neutralizes the hemolytic activity of the streptococcal exotoxin and streptolysin O at very low concentrations. Moreover, the inhibition of 96% was obtained at 1:262144 DE dilution. The inhibitory substance was found to be steroidal in nature and not proteinaceous as deproteinization of DE did not decrease its inhibitory effect. The results indicated that the neutralization property of this factor is most probably due to erythrocyte membrane stabilization and inhibition of streptolysin O enzyme. Date intake did not affect the titer of anti-streptolysin O antibodies according to a study conducted by El-Mougy, Abdel-Aziz (7).

The antibacterial activity of aqueous and ethanol extracts of *P. dactylifera* fruits against *Escherichia coli*, *Salmonella enterica*, and *Bacillus subtilis* was tested using a disc diffusion method, while *Staphylococcus aureus* and *Enterococcus faecalis* were mildly inhibited. It is revealed that the presence of esculetin tannic acid is responsible for this antibacterial effect (8). Therefore, the current study aimed to evaluate the effects of DE on the hematological and biochemical parameters in rabbits.

2. Materials and Methods

2.1. Plant Materials

Date fruits were purchased from the markets of Al-Diwaniyah, Iraq. Prior to the pit separation, the dates were washed carefully with double distilled water (DDW) at room temperature. The dates' pits were manually separated from the fruits, soaked completely in cold DDW (2:4 W/V), and left for 72 h at 8˚C. Dried dates were then grounded into a fine powder and immersed in cold DDW (2:42:4 W/V) for 72 h at 8˚C. The water extract was freshly prepared.

2.2. Animals

New Zealand white rabbits (Faculty of Veterinary Medicine, Experimental Animal House Center, Iraq) were housed under controlled environmental conditions (20±2˚C,14:10 h light: dark cycle, 55% humidity) and allowed *ad libitum* access to DE (treated group) or chow (control) and water. The mean age of the animals was 2 months, and they weighed 500-700 g. The rabbits' weighting was done on the first and last day of the study. In total, 30 rabbits were used in this investigation, and they were divided into two groups (n=15). Group one (G1) (control group) received only a meal without dates for 30 days, and group two (G2) was given date seed extract about 30 ml/kg b.w. for 30 days. International laws and policies were committed in procedures that involved rabbits' care.

The animals were anesthetized by an injection of 0.25 ml of ketamine (15 mg/kg b.w.): xylazine (10 mg/kg b.w.) mixture into the marginal ear vein. Blood was aspirated from the rabbit's heart with Venoject (BD Life Sciences, Cockeysville, Md, USA) without any anticoagulants. The amount of blood collected from each doe was about 100 ml. The blood samples were centrifuged at 3000×g for 10 min. After centrifugation, the supernatant was carefully separated and filtered under sterile conditions, and then stored at -20˚C for later use (9).

2.3. Complete Blood Count Parameters

Appropriate techniques were used to assess hematological parameters. The Cyano-methemoglobin method (7) was employed to measure Hgb concentration. The packed cell volumes (PCV) were evaluated using just a microhematocrit centrifuge (8). Red blood cells (RBC) and white blood cells (WBC) were measured under a high-powered microscope using a double-improved Neubauer counting chamber (10) and a high-powered microscope that used a double-improved Neubauer counting chamber (11). Furthermore, the below-mentioned equations (12) were

used to calculate Hgb indices, including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentrations (MCHC) (13).

MCH=HB×10/TRBCs Count, MCH=HB×100/HCT, MCV=HCT×10/TRBCs COUNT.

2.4. Biochemical Parameters

The levels of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined using kinetic kits according to a method by Ahmed, Ahmed (14); moreover, protein concentration was determined using a method by Pilaski (15). Furthermore, Patton's method (16) was used to calculate urea, whereas creatinine was calculated using a method by Provan, Singer (17).

2.5. Statistical Analysis

The results were statistically presented as mean±SE. The t-test was used to compare the values of the G1 and G2 by SPSS software (version 19) (18).

3. Results

The recorded data showed a significant increase ($P \leq 0.05$) in Hgb concentrations (Hgb, MCV, MCH, MCHC, and PCV) in G2, compared to G1. On the other hand, the results showed a significant difference between G1 and G2 in terms of RBCs, WBCs, and platelet count (PLT) ($P \leq 0.05$; Table 1). Additionally, date caused a significant increase in AST, ALT, and ALP levels ($P \leq 0.05$) in the serum of rabbits treated with date, compared to the controls. Furthermore, a significant increase in creatinine, blood urea, and nitrogen were recorded in G2, compared to G1. However, there were no significant changes in total protein in G2, compared to G1, after date nourishing.

Table 1. Hematological and biochemical analysis early in the morning and after date feeding (n=15) (mean±SE) in rabbits

Blood parameters	G1 (Early in the morning)	G2 (Early in the morning)	G2 (after date feeding)
MCH pg	18.5±0.5	18.9±0.4	23.7±0.8
MCHC %	27.8±0.5	25±0.6	33.5±o.6
MCV fl	56±0.4	50±0.7	60.3±0.7
Hgb g/dl	9.7±0.2	9.5±0.7	14.2±0.2
HCT%	32.1±0.3	31±0.4	40±0.6
WBCs×10^3/µl	4.56±0.1	5.7±0.5	7.9±0.4
RBCs×10^6/µL	4.89±0.7	5.3±0.4	6.31±0.5
PLT×10^3/µL	321±0.3	336±0.6	435±0.6
AST U/l	7.9±0.3	6.3±0.7	10..4±0.4
ALT U/l	6.3±0.5	5.7±0.2	9.41±0.5
ALP U/L	13.8±0.2	12.8±0.7.	22.9±0.3
Creatinine mmol/L	0.03±0.27	0.07±0.1	0.11±0.1
BUN mmol/L	10.8±0.5	11.9±0.3	14.9±0.5
Albumin g/l	29.6±0.3	25.9±0.2	33.2±0.6
Total protein	5.9±0.2	6.2±0.6	8.9±0.5

4. Discussion

Hgb is the major component of red blood cells, and a high Hgb content means that the oxygen-carrying protein Hgb is present in excess in the blood. Since each cell may not have the same quantity of Hgb proteins, a high Hgb concentration differs from a high RBC count. As a result, even if your RBC count is normal, you could have a high Hgb count.

Date causes a substantial rise ($P \leq 0.05$) in Hgb concentration, MCH, and MCHC. Moreover, dates lead to a significant difference in PCV percent, WBC, and RBC, in the treated group, compared to the control group.

Our findings demonstrated that dates produce a substantial ($P \leq 0.05$) elevation in total protein, as well as ALT, AST, and ALP; however, no significant

difference was found between the date-treated and control groups regarding albumin. Although ALT is more specific to the liver than AST, they are both considered to be two of the most significant tests for detecting liver impairment. Lower release of tissue-specific enzymes and other intracellular proteins as a result of oxidative stress during metabolism could explain the drop in serum total protein and ALT. The method by which dates produce their hepatoprotective effects is unknown. However, it is probable that 0.137% vitamin C found in date has a role in hepatoprotection (19). Creatinine is a naturally occurring chemical produced by the body's metabolism. Dates cause a substantial increase ($P \leq 0.05$) in creatinine, compared to the control group, according to the findings of this study (Table 1). The ability of dates to promote the filtration process and increase the efficacy of the two kidneys may be the blame for this.

5. Conclusion

According to the current findings, dates are among the most important fruits that increase body activities. Moreover, dates have positive effects on most of the liver enzymes and complete blood count parameters. Additionally, they have a significant effect on kidney parameters in rabbits when used as nutrition in the evening, compared to the early morning (on an empty stomach after overnight fasting).

Authors' Contribution

Study concept and design:
Acquisition of data:
Analysis and interpretation of data:
Drafting of the manuscript:
Critical revision of the manuscript for important intellectual content:
Statistical analysis:
Administrative, technical, and material support:

Ethics

International laws and policies were committed in procedures that involved rabbits' care and was approved by the ethics committee of the University of Al-Qadisiyah, Al Diwaniyah, Qadisiyyah Province, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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