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## **Chemical Composition and Antioxidant Activity of Dates and Dates-Camel-Milk Mixtures as a Protective Meal against Lipid Peroxidation in Rats**

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**Abstract:** Dates and camel milk have been reported to be used for prevention and treatment of various diseases in Arab countries. This study was designed to investigate the antioxidant activity of dates and camel milk, alone or as a mixture and their effect in lead acetate-poisoned rats. Lead was administered to rats in drinking water at a concentration of 0.6% to generate lipid peroxidation. Three cultivars of date fruits grown locally (Nabtat Ali, Sukkari and Rashudia) were tried when mixed individually with camel milk against lipid peroxidation generated by lead. Chemical composition of dates, camel milk and their mixture was determined. The organoleptic evaluation of palatability of date-camel milk mixtures was performed. Serum glucose, triglycerides and urea concentrations and activities of Aspartate Aminotransferase (AST/GOT), Alanine Aminotransferase (ALT/GPT) and Glutathione-S-Transferase (GST) enzymes were determined. Drinking water containing 0.6% lead acetate resulted in significant increase in serum glucose, triglycerides and urea concentrations, as well as increased activity of serum enzymes were investigated. It also reduced the serum activity of the antioxidant enzyme Glutathione-S-transferase. Mixing camel milk with date varieties of Rashudia, Nabtat Ali or Sukkari in ratio of 2:1 (v/w) increased milk antioxidant capacity by 49.2, 62.8 and 81.3%, respectively. It also reduced the elevated activities of serum enzymes, the concentration of urea and increased the activity of Glutathione-S-Transferase enzyme in poisoned rats. Mixtures of camel milk with the different cultivars of dates produced different beneficial effects. In conclusion, feeding a mixture of camel milk with dates seems to enhance the body defense against free radicals generated by the lead acetate poisoning mainly due to the high contents of phenolics and flavonoids in the mixture.

**Key words:** Dates, antioxidant activity, camel milk, Saudi Arabia, Nabtat Ali, Rashudia, Sukkari types, phenolics, flavonoids

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### **INTRODUCTION**

Date palm (*Phoenix dactylifera* L.) fruits is an important component of the diet in most of the hot arid and semi arid regions of the world. Date palm fruits were found to contain

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carbohydrates (44-88%), fats (0.2-0.4%), proteins (2.3-5.6%), fibers (6.4-11.5%), minerals and vitamins (Al-Shahib and Marshall, 2003). Carbohydrates in dates are mostly in the form of fructose and glucose, which are easily absorbed by the human body (Al-Farsi *et al.*, 2005). Interestingly, dates contain higher concentrations of proteins when compared to other cultivars of fruits such as apples, oranges, bananas and grapes (containing 0.3, 0.7, 1.0 and 1.0% proteins, respectively) (Al-Showiman, 1998).

Twenty-three different amino acids were found in dates proteins, many of which are not found in the most popular fruits (Al-Shahib and Marshall, 2003). Several studies in the literature concluded that the aqueous extracts of dates have potent antioxidant and antimutagenic activity (Mansouri *et al.*, 2005; Mohamed and Al-Okabi, 2004). Dates was reported to have the second highest antioxidant activity among 28 fruits commonly consumed in China (Guo *et al.*, 2003). Antioxidants have received increased attention by nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative diseases such as cancer, cardiovascular diseases and aging (Kaur and Kapoor, 2001; Young and Woodside, 2001). The most effective antioxidants in this respect appear to be the flavonoids and phenolics. Because of their metal-chelating and radical-scavenging properties, phenolics were considered effective inhibitors of lipid peroxidation (Mansouri *et al.*, 2005). Al-Farsi *et al.* (2005) found that dates were a high source of antioxidants, anthocyanins, carotenoids and phenolics. Camel milk was claimed by some locals in the Arabic world to have protective health and therapeutic values. Clinical trials have shown that the daily consumption of 0.5 L of camel milk reduced the need for insulin medication by an average of 30% in patients with type-1 diabetes with better control of blood glucose and lipids levels (Agrawal *et al.*, 2002, 2005). It has been concluded that a mixture of camel milk and dates might prove to be beneficial for the treatment of various ailments (Agrawal *et al.*, 2005).

This study was designed to investigate the antioxidant activity of dates and camel milk, alone or as a mixture and their effect in lead acetate-poisoned rats.

## **MATERIALS AND METHODS**

This study was carried out in the College of Agriculture and Veterinary Medicine in 2008/2009.

### **Materials**

#### **Dates**

Three cultivars of popular date fruits (called Nabtat Ali, Sukkari and Rashudia) commonly grown in Qassim region were purchased from the local date market in Buraidah, Qassim, Saudi Arabia.

#### **Camel Milk**

Camel milk was obtained from healthy lactating she-camels kept at the Experimental Research Station of the College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia.

#### **Dates and Camel Milk Mixed Ingredient**

Camel milk was heated at 85°C for 15 min, cooled rapidly to 4°C to kill pathogens and then mixed with dates (after removing the seeds) in 2:1 (v/w) ratio (Camel milk: dates). The mixture was then homogenized with an electric blender and stored in a refrigerated temperature at 4°C.

### **Animals**

Thirty five male Swiss albino rats weighting about 120-150 g were randomly divided into five groups of 7 rats each. The rats of each group were placed in a cage and given the basal diets for one week as an adaptation period. The animals were handled and treated with the compliance with the University of California, San Francisco Occupational health and safety in the care and use of research animal.

### **Diet**

The composition of basal diet used in this study was as follows: milk protein (12%), sucrose (5%), fat (10%), vitamin mixtures (1%), salt mixtures (4%), fiber (4%) and starch (64%). The basal diet was mixed with dates-camel milk mix in 3:1 ratio.

Lead acetate was administered in drinking water at a concentration of 0.6%. Group 1 rats were fed on the basal diet only (negative control). Group 2 rats were fed on the basal diet+lead acetate in drinking water (positive control). Group 3 rats were fed on basal diet+mixture of Rashudia dates-camel milk mixture+lead acetate (0.6%) in drinking water. Group 4 rats were fed on basal diet+mixture of Nabtat Ali dates-camel milk mixture+lead acetate (0.6%) in drinking water. Group 5 rats were fed on basal diet+Sukkari dates-camel milk mixture+lead acetate (0.6%) in drinking water.

In groups 3, 4 and 5, fed with dates-camel milk mixture started three weeks prior to administration of lead acetate in drinking water and continued for another four weeks. At the end of the experimental period, rats were anesthetized by diethyl ether, blood samples collected and then rats sacrificed. Blood samples were taken into plain tubes to obtain serum. Serum was frozen at -20°C until analyzed.

### **Methods**

#### **Sugars**

To 0.01 g of date flesh, 40 mL of ethanol: water (50% v/v) extraction solution was added. The mixture was sonicated for 15 min at 60°C and cooled at room temperature. The solution was then completed to 50 mL total volume with the extraction solution and filtered through 0.43 µm filter. The concentrations of glucose, fructose, maltose, sucrose and lactose were read in HPLC (Shimadzu, Model 2001, Japan).

#### **Amino Acids**

Determination of amino acids composition of dates in this experiment was carried out according to the method described by Kondo *et al.* (1984) using a HPLC (Shimadzu, Model 2001, Japan).

#### **Antioxidants**

Total phenolics were determined according to the method of Makkar (2003). Total flavonoids were estimated by the method of Kim *et al.* (2006). Antioxidant activity (DPPH methods) was determined according to the method of Sanchez-Moreno *et al.* (1998). Antioxidant capacity was quantified as µg Trolox equivalents using a standard curve.

#### **Chemical Composition**

Chemical composition of dates and camel milk mixture was performed by conventional Proximate Analysis Techniques.

### Serum Constituents

Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) activities were determined according to the method of Reitman and Frankel (1957). Urea were determined in serum according to the methods of Tietz (1987). Triglycerides were determined in serum according to the methods of Stein and Myers (1995). Blood glucose concentration was determined by the Haemo-Glukotest 20-800-R and measurements read with a Reflolux test (Boehringer-Mannheim) (Brodrick *et al.*, 1987).

Activities of Glutathione-S-Transferase (GST) activity was determined according to Habig *et al.* (1974).

### Organoleptic Evaluation

The organoleptic evaluation of palatability of date-camel milk mixtures was performed by panel from the staff of the Department of Food Science and Human Nutrition. That evaluation was for appearance, color and flavor and results were expressed as: (+++) very good, (++) good, (+) acceptable, (-) unacceptable, as described by NASA (1999).

### Statistical Analysis

Results are described as Mean±SD. Statistical analysis of differences between means was conducted according GLM procedure of SAS program (SAS, 1996).

## RESULTS

### Sugar Contents of Dates

As shown in Table 1 the highest level of glucose and fructose were reported in Rashudia cultivars, while the content of sucrose was highest in Sukkari cultivars.

The contents of glucose in Sukkari, Nabtat Ali and Rashudia dates were 12.45, 19.0 and 26.59%, respectively. The content of sucrose was highest in the Sukkari date (35.94%) followed by Nabtat Ali (28.84%) and Rashudia date (9.27%).

### Amino Acids Content of Dates

Fifteen amino acids were detected in the three dates cultivars. Sukkari cultivars contains high levels in Aspartic acid, glutamic acid, glycine, leucine, isoleucine, histadine, lysine, arginine followed by Rashudia cultivars (Table 2).

### Antioxidants Activity (DPPH), Phenolics and Flavonoids Contents of the Tested Dates Cultivars, Camel Milk and Their Mixtures

Antioxidant activity was found to be highest in Rashudia (4935±329 µmol Trolox equivalents/100 g sample) followed by Nabtat Ali and then Sukkari cultivars. Camel milk contained only 662±11 µmol Trolox (Table 3). Antioxidant activity in Mixtures of camel milk and Rashudia, Nabtat Ali and Sukkari was found to be 988±87; 1087±38 and 1200±38 µmol Trolox equivalents/100 g sample, respectively.

Table 1: Sucrose, glucose, fructose and moisture content in Nabtat Ali, Rashudia and Sukkari dates cultivars (%)

Content	Rashudia	Nabtat Ali	Sukkari
Fructose	23.39	15.92	9.17
Glucose	26.59	19.00	12.45
Sucrose	9.27	28.84	35.94
Moisture	27.20	24.85	19.82

Table 2: Amino acids contents in Rashudia, Nabtat Ali and Sukkari dates cultivars (%)

Amino acids	Rashudia	Nabtat Ali	Sukkari
Aspartic acid	0.25	0.17	0.34
Threonine	0.12	0.08	0.10
Serine	0.14	0.11	0.13
Glutamic acid	0.31	0.21	0.49
Glycine	0.15	0.11	0.17
Alanine	0.15	0.11	0.16
Valine	0.13	0.09	0.14
Methionine	0.03	0.04	0.06
Isoleucine	0.07	0.07	0.10
Leucine	0.17	0.14	0.20
Tyrosine	0.06	0.05	0.08
Phenylalanine	0.14	0.09	0.15
Histadine	0.08	0.06	0.10
Lysine	0.03	0.02	0.07
Arginine	0.03	0.02	0.07

Table 3: Antioxidant activity, total phenolics and total flavonoids content of the tested dates, camel milk and their mixtures (Mean±SD)

Trait	Antioxidant activity µmol Trolox equivalents/100 g sample	Total phenolics mg gallic acid equivalents/100 g sample	Total flavonoids mg quercetin equivalents/100 g sample
Rashudia	4935±329 <sup>a</sup>	110.50±3.5 <sup>a</sup>	17.1±0.95 <sup>a</sup>
Nabtat Ali	4747±329 <sup>a</sup>	104.00±6.0 <sup>a</sup>	14.7±0.95 <sup>a</sup>
Sukkari	3603±197 <sup>b</sup>	88.00±9.3 <sup>b</sup>	11.3±1.03 <sup>b</sup>
Camel milk	662±11 <sup>d</sup>	16.53±2.2 <sup>d</sup>	ND
Rashudia and camel milk mixture	988±87 <sup>c</sup>	54.00±3.5 <sup>c</sup>	4.2±0.46 <sup>c</sup>
Nabtat Ali and camel milk mixture	1087±38 <sup>c</sup>	60.00±1.6 <sup>c</sup>	3.7±0.90 <sup>c</sup>
Sukkari and camel milk mixture	1200±38 <sup>c</sup>	56.00±1.5 <sup>c</sup>	3.9±0.64 <sup>c</sup>

ND: Not detected. Means in the same column with different superscript are significantly different (p<0.05)

Table 4: Chemical composition of dates cultivars, camel milk and their mixtures (%)

Composition	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>
pH	5.92	6.99	7.24	6.73	6.37	6.57	6.75
Moisture	15.50	18.95	13.82	87.90	70.81	67.66	67.74
Dry matter	84.50	81.00	86.10	12.10	29.19	32.34	32.26
Proteins	0.89	1.00	0.93	3.21	1.93	2.65	2.19
Carbohydrates	58.86	63.86	67.56	4.20	20.59	22.12	23.24
Ash	1.40	1.25	1.52	1.09	2.72	2.45	2.42

T<sub>1</sub>: Rashudia, T<sub>2</sub>: Nabtat Ali, T<sub>3</sub>: Sukkari, T<sub>4</sub>: Camel milk, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub>: Mixture of camel milk with Rashudia, Nabtat Ali and Sukkari, respectively

The antioxidant activity of Rashudia and Nabtat Ali cultivars was significantly (p<0.05) higher than antioxidant activity in Sukkari cultivars. Mixing camel milk with each date cultivars increased (p<0.05) significantly the antioxidant activity of camel milk.

The three tested dates cultivars were found to be rich in phenolics and flavonoids (Table 3). Total phenolics in dates cultivars were significantly (p<0.05) higher than the phenolic contents in camel milk. Mixing dates cultivars with camel milk increased significantly (p<0.05) the phenolic contents in camel milk.

Total flavonoids were detected in small quantities in dates. Rashudia and Nabtat Ali contain a significantly (p<0.05) higher amount of flavonoids when compared with Sukkari cultivars. A slight increase in flavonoids contents in camel milk was noticed when mixed with dates cultivars (Table 3).

### **Chemical Composition of Dates, Camel Milk and Their Mixtures**

The concentrations of carbohydrates, proteins and total dry matter were found to be highest in Sukkari cultivars (Table 4).

Table 5: Organoleptic evaluation of date-camel milk mixtures

Sensory evaluation	Rashudia and camel milk mixture	Nabtat Ali and camel milk mixture	Sukkari and camel milk mixture
Appearance	+++	++	++
Color	++	+	++
Flavor	++	++	+++
Odor	++	++	++
Overall	++	++	++

+++; Very good, ++: Good, +: Accepted, -: Un-accepted

Table 6: Effect of feeding of dates and camel milk mixtures on serum glucose, triglyceride and urea concentration (Mean±SD)

Trait	Glucose	Triglyceride (mg dL <sup>-1</sup> )	Urea
Negative control	99.69±0.54 <sup>c</sup>	74.63±1.04 <sup>d</sup>	49.80±2.36 <sup>b</sup>
Positive control	129.33±4.04 <sup>b</sup>	178.66±1.52 <sup>a</sup>	73.00±6.55 <sup>a</sup>
Rashudia and camel milk mixture	123.66±4.61 <sup>b</sup>	81.56±3.60 <sup>b</sup>	38.23±1.18 <sup>c</sup>
Nabtat Ali and camel milk mixture	85.00±4.25 <sup>d</sup>	77.33±1.19 <sup>cd</sup>	37.16±3.70 <sup>c</sup>
Sukkari and camel milk mixture	153.0±10.39 <sup>a</sup>	80.66±1.15 <sup>cb</sup>	36.26±0.70 <sup>c</sup>

ND: Not detected. Means with different superscript are significantly different (p<0.05)

Table 7: Effect of feeding dates and camel milk mixtures on serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and glutathione-S-transferase (GST) activity (Mean±SD)

Trait	ALT (U L <sup>-1</sup> )	AST	GST activity (%)
Negative control	151.12±9.42 <sup>d</sup>	191.86±1.34 <sup>b</sup>	16.94±0.80 <sup>c</sup>
Positive control	287.00±11.78 <sup>a</sup>	219.80±6.38 <sup>a</sup>	11.21±0.51 <sup>d</sup>
Rashudia and camel milk mixture	196.52±13.09 <sup>c</sup>	181.97±2.75 <sup>c</sup>	20.48±0.60 <sup>a</sup>
Nabtat Ali and camel milk mixture	219.22±6.61 <sup>b</sup>	135.99±5.20 <sup>c</sup>	18.55±0.72 <sup>a</sup>
Sukkari and camel milk mixture	212.82±0.01 <sup>cb</sup>	169.94±6.13 <sup>d</sup>	19.03±0.21 <sup>a</sup>

Means with different superscript are significantly different (p<0.05). ND: Not detected

### Organoleptic Evaluation of Dates-Camel Milk Mixtures

Results of palatability of dates-camel milk mixtures are shown in Table 5. Camel milk when mixed with either dates cultivars was found acceptable by the test panel.

### Serum Constituents

There was a significant increase in the concentration of serum glucose, triglycerides and urea following poisoning with lead acetate (Table 6).

Feeding with a mixture of Nabtat Ali and camel milk resulted in significant (p<0.05) lowering of blood glucose and triglycerides levels in the poisoned rats (Table 6). The three cultivars of dates-camel milk mixtures significantly lowered (p<0.05) serum urea levels in the poisoned rats (Table 7).

Feeding lead acetate also increased significantly (p<0.05) the activity of AST enzyme in serum (Table 7). Feeding camel milk mixture with Nabtat Ali date, Rashudia date or Sukkari date resulted in a significant decrease in the activity of this enzyme in serum.

Feeding lead acetate to rats significantly increased the activity of ALT in their serum (Table 7). Feeding of camel milk with Rashudia date decreased significantly (p<0.05) the activity of ALT.

Feeding rats with lead acetate resulted in a significant (p<0.05) decrease in the activity of glutathione-S-transferase enzyme in serum (Table 7). Feeding with camel milk-dates mixtures was associated with a significant (p<0.05) serum increase in the activity of this enzyme. The highest increase in enzyme activity was obtained with Rashudia date-camel milk mixture followed by Sukkari and then Nabtat Ali date mixtures (Table 7).

## **DISCUSSION**

Earlier studies have shown that lead acetate poisoning provoked generation of free radicals that induced tissue damage (Mousa *et al.*, 2002; Al-Wabel *et al.*, 2007). This damage was mediated by two mechanisms, generation of Reactive Oxygen Species (ROS) and by direct depletion of antioxidant reserves.

In the present study, date fruits were found to be rich in total phenolics and total flavonoids. The highest antioxidant activity was observed in Rashudia date followed by Nabtat Ali and finally in Sukkari dates. Similar results have been documented by Al-Farsi *et al.* (2007). Antioxidant activity of dates has been observed previously in Algerian dates (Mansouri *et al.*, 2005), Omani dates (Al-Farsi *et al.*, 2005), Saudi Arabian dates (Al-Laith, 2007), Chinese dates (Guo *et al.*, 2003) and Iranian dates (Biglari *et al.*, 2008). The phenolics and flavonoids contents of the date fruit were considered to be the main contributors to its antioxidant activity (Mansouri *et al.*, 2005; Al-Laith, 2007).

Camel milk has powerful antibacterial properties which could modulate the immune system. Drinking non-pasteurized camel milk has been observed to be beneficial to people with infection of the alimentary canal and autoimmune diseases (Shabo *et al.*, 2008). The camel milk was found to be low in cholesterol and glucose content, high in minerals (sodium, potassium, iron, copper, zinc and magnesium) and high in vitamin C (Mohamed *et al.*, 2005) content.

Camel milk was found in this study to have low concentration of phenolics and flavonoids. Addition of dates to milk increased the total phenolic diet and it reached maximum values when milk mixed with Nabtat Ali dates.

Drinking water containing lead acetate resulted in a significant decrease in the activities of serum glutathione-S-transferase enzyme. The later is a family of enzymes that utilize glutathione in reactions leading to the detoxification of a wide range of harmful compounds, including carcinogens, therapeutic drugs and products of oxidative stress. Reduced serum activity of this enzyme is an indication of depleted glutathione level.

Probably the body will sort out this deficiency by using alternative defense mechanisms. Using the phenolics and flavonoids compounds in camel milk and dates mixture could significantly contribute to defense against oxidative stress.

The AST is an enzyme found primarily in the liver, heart, kidney, pancreas and muscles. Activity of AST increases in serum following tissue damage. Feeding rats with lead acetate increased the activities of this enzyme in serum as a result of organs damage by peroxides generated by lead (Mousa *et al.*, 2002). Ingestion of Nabtat Ali dates and camel milk mixture or Rashudia dates and camel milk mixture resulted in a significant decrease in the activity of this enzyme in serum indicating their protective role against tissue peroxidation. Ingestion of Sukkari dates and camel milk mixture had no significant reduction effect on the activity of this enzyme.

## **CONCLUSION**

In conclusion a mixed meal of dates and camel milk might play a protective role against tissue damage mediated by free radicals. All varieties of dates could serve as a good source of natural antioxidants; however our observation indicated that the best combination with camel milk as a source of natural flavonoids and phenolics would be a mixture of Nabtat Ali or Rashudia dates.



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