

# Protective Effect of Extracts from Dates (*Phoenix dactylifera* L.) on Carbon Tetrachloride–Induced Hepatotoxicity in Rats

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## ABSTRACT

The ameliorative activity of aqueous extracts of the flesh and pits of dates (*Phoenix dactylifera* L.) on carbon tetrachloride (CCl<sub>4</sub>)-induced hepatotoxicity was studied in rats. Sixty male Wistar rats were divided into six equal groups of 10. Four groups received extracts of flesh or pits of *Phoenix dactylifera* and intraperitoneal (IP) CCl<sub>4</sub> (0.2 ml/100 g) either before or after administration of flesh or pits. Two groups were controls, one treated with CCl<sub>4</sub> and one with only saline. Liver damage was assessed by liver morphology, histology, and estimation of plasma concentration of bilirubin and enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase. Treatment with aqueous extract of date flesh or pits significantly reduced CCl<sub>4</sub>-induced elevation in plasma enzyme and bilirubin concentration and ameliorated morphological and histological liver damage in rats. This study sug-

gests that CCl<sub>4</sub>-induced liver damage in rats can be ameliorated by treatment of extracts from date flesh or pits.

## INTRODUCTION

For centuries, *Phoenix dactylifera* (Palmae) has been used in the Middle East as a staple food. No fewer than 800 uses are recorded for the date palm.<sup>1</sup> The Palm family is a symbol of prosperity and love to Muslims and its legend dates back to Judeo-Christian mythology. In local medicinal practices, dates are considered a tonic. Some consider it to be an aphrodisiac. The flower of the plant is used as a purgative.<sup>2</sup>

Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle-stimulating hormone, and luteinizing hormone in rats.<sup>1</sup> The pollen grains of date palm have been used by Egyptians to improve fertility in women.<sup>3</sup> Date pits have been included in animal feed to enhance growth, an action that has been ascribed to an increase in the plasma level of estrogens<sup>4</sup> or testosterone.<sup>5</sup>

Many Middle Easterners believe that consumption of dates, particularly in the morning on an empty stomach, can reverse the actions of any toxic material that the subject may have been exposed to. Therefore, we sought to assess the ability of date flesh and pits to treat or prevent some of the toxic actions of carbon tetrachloride (CCl<sub>4</sub>) on the liver in rats. The latter is a model for acute viral hepatitis.<sup>6</sup>

## MATERIALS AND METHODS

### Plant Material

Date fruits were obtained from the Al-Gaseem Date Factory in the central region of the Kingdom of Saudi Arabia. The flesh was manually separated from the pits and soaked in cold distilled water (1:3 ratio, weight to volume) and kept for 48 hours at a temperature of 4°C. The pits were rinsed clear of any flesh and dried at room temperature. The dried pits were then ground into a fine powder and immersed in cold distilled water (1:3 ratio, weight to volume) for 48 hours at a temperature of 4°C. The water extract was prepared freshly and given to the animals ad libitum in place of rat chow.

### Animals

Male Wistar rats, 8 to 9 weeks of age and weighing 180 to 200 g, were obtained from the Experimental Animal House Center, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia. All animals were given food (rat chow or date extract) and water ad libitum, and were maintained at a relative humidity of 65% to 86%, a temperature of 23°C to 25°C, and in a schedule of 12 hours of light and 12 hours of dark (lights on at 0600). Rats were weighed at the beginning and end of the study. Procedures involving animals and their care were conducted in conformity with international laws and policies.

### Chemicals

High-grade chemicals were obtained from Sigma-Aldrich and included CCl<sub>4</sub>, ether, absolute ethanol, xylol, methyl benzoate, hematoxylin, eosin red, paraffin (melting point, 60°C–70°C), and beeswax.

## Assessment of Hepatoprotective Activity

Sixty animals were randomly divided equally into six groups of 10:

**Group 1** (controls): receiving normal saline orally (0.2 ml/100 g) for 16 consecutive days.

**Group 2** (pretreatment experiment-*flesh*): allowed free access to aqueous extract of date flesh ad libitum for 28 consecutive days and treated with intraperitoneal (IP) CCl<sub>4</sub> on Days 14, 15, and 16 of the treatment period.

**Group 3** (post-treatment experiment-*flesh*): given aqueous extract of date flesh ad libitum for 14 consecutive days and treated with IP CCl<sub>4</sub> on Days 1, 2, and 3 of the treatment period.

**Group 4** (pretreatment experiment-*pits*): allowed aqueous extract of date pits ad libitum for 28 consecutive days and treated with IP CCl<sub>4</sub> on Days 14, 15, and 16 of the treatment period.

**Group 5** (post-treatment experiment-*pits*): given aqueous extract of date pits ad libitum for 14 consecutive days and treated with IP CCl<sub>4</sub> on Days 1, 2, and 3 of the treatment period.

**Group 6** (CCl<sub>4</sub>-treated control): injected with a fresh mixture of equal volumes of CCl<sub>4</sub> and olive oil by three IP injections at doses of 0.2 ml/100 g body weight.

Twenty-four hours after the last treatment (Day 16 or Day 29) the rats were euthanized. Hepatoprotective activity was calculated according to the formula of Singh et al:<sup>7</sup>

$$\text{Hepatoprotective activity (\%)} = 1 - \frac{(\text{PC} - \text{S})}{(\text{C} - \text{S})} \times 100$$

where PC, C, and S are the measurable variables in rats treated with date flesh or pits plus CCl<sub>4</sub>, CCl<sub>4</sub>, and saline-treated animals, respectively.

### Blood Sampling

Blood was collected in heparinized tubes from the inner canthus on the 29<sup>th</sup> day or the 16<sup>th</sup> day in the pre- or post-treated groups, respectively. Plasma was separated by cen-

**Table 1.** Effect of Pre- and Post-treatment with Aqueous Date Flesh and Pits on CCl<sub>4</sub>-Induced Liver Damage in Wistar Rats

Treatment	Aspartate aminotransferase (U/L)	Alanine aminotransferase (U/L)	Alkaline phosphatase (U/L)	Bilirubin (μmol/L)
Control	106.8 ± 3.29	34.62 ± 1.34	155.0 ± 2.54	0.17 ± 0.03
Date flesh pre-treatment	136.6 ± 3.12	46.6 ± 1.77 <sup>†</sup>	180.4 ± 1.97 <sup>†</sup>	0.19 ± 0.01
Date flesh post-treatment	124.4 ± 4.27 <sup>†</sup>	43.68 ± 0.64 <sup>†</sup>	192.4 ± 4.72 <sup>†</sup>	0.20 ± 0.01
Date pits pre-treatment	162.0 ± 4.64 <sup>†</sup>	59.80 ± 1.58 <sup>†</sup>	181.4 ± 3.17 <sup>†</sup>	0.21 ± 0.01
Date pits post-treatment	158.4 ± 6.43 <sup>†</sup>	57.40 ± 1.34 <sup>†</sup>	172.2 ± 10.33	0.20 ± 0.00
Carbon tetrachloride	282.6 ± 2.96 <sup>†</sup>	85.6 ± 1.99 <sup>†</sup>	253.4 ± 5.44 <sup>†</sup>	3.29 ± 0.06 <sup>†</sup>

Data are expressed as mean ± SE (n = 10)

<sup>†</sup>Significantly different than control at *P* < .01.

**Table 2.** Hepatoprotective Activity of Date Flesh and Pits in CCl<sub>4</sub>-Induced Hepatotoxicity in Wistar Rats

Clinical Chemistry Liver Function Indicator	Date Flesh		Date Pits	
	Pre-treatment (% protection)	Post-treatment (% protection)	Pre-treatment (% protection)	Post-treatment (% protection)
Aspartate aminotransferase	83.05	89.99	68.60	70.65
Alanine aminotransferase	76.50	82.23	50.61	55.32
Alkaline phosphatase	74.19	61.99	73.17	82.52
Bilirubin	99.36	99.04	96.00	99.04

$$\% \text{ Protection} = 1 - \frac{(PC - S)}{(C - S)} \times 100$$

where PC, C, and S are the measurable variables in rats treated with date flesh or pits plus CCl<sub>4</sub>, CCl<sub>4</sub>, and saline-treated animals, respectively.

trifugation at 900 g for 10 minutes at 4°C, and used for measuring the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin concentration using the Boehringer Mannheim/Hitachi autoanalyzer-902.

Liver morphology was assessed by an observer blinded to the treatments. Pieces of the same lobe of liver from each animal were taken and dipped in formol-saline. The fixed tissue was cut into 5-μm sections, processed, and stained with hematoxylin and eosin. Liver sections were analyzed by an examiner blinded to the treatments.

### Statistical Analysis

Values reported are means ± SE (n = 10). Experimental results were statistically analyzed using the Student's *t*-test for unpaired data, with *P* value of less than .05 considered significant.

## RESULTS

The CCl<sub>4</sub>-treated animals exhibited a significant increase (*P* < .01) in plasma enzyme activity and bilirubin concentration compared with saline-treated control rats (Table 1). A significant reduction was found in elevated AST, ALT, and ALP values in rats subjected to both pre- and post-treatments with the aqueous extracts of both date flesh and date pits. Liver enzyme values were higher in the four experimental groups than in the saline-treated controls, but the liver enzyme values were decreased to about half of those found in CCl<sub>4</sub>-treated control animals for all liver function tests except bilirubin.

Expressed in percentage of protection provided, both date palm flesh and pits given pre- or post-treatment were hepatoprotective, as calculated by the formula of Singh et al.<sup>7</sup> Table 2 presents the results of our calculations of hepatoprotection as pro-

vided by date palm flesh and pits in CCl<sub>4</sub>-induced toxicity in rats.

## DISCUSSION

Liver cirrhosis induced by CCl<sub>4</sub> is perhaps the best-studied model of liver cirrhosis.<sup>8</sup> Several mechanisms underlying this toxicity have been suggested.<sup>9</sup> The reduction of CCl<sub>4</sub>-induced elevated plasma activities of AST, ALT, ALP, and bilirubin level in animals pre- and post-treated with the aqueous extracts of date flesh or pits shows their ability to restore the normal functional status of the poisoned liver, and also to protect against subsequent CCl<sub>4</sub> hepatotoxicity.

The mechanism by which the date pits and flesh induces its hepatoprotective activity is not certain. However, it is possible that  $\beta$ -sitosterol, a constituent of *Phoenix dactylifera*,<sup>1</sup> is at least partly responsible for the protective activity against CCl<sub>4</sub> hepatotoxicity.<sup>10</sup> CCl<sub>4</sub>, the inactive metabolite, is transformed to a free radical through the microsomal cytochrome P-450-dependent enzyme, resulting in activation of CCl<sub>4</sub> toxicity. An additional and important factor in the hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P-450, thereby favoring liver regeneration. On that basis, it is suggested that flavonoids in *Phoenix dactylifera* could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase.<sup>11</sup> In addition, the recorded content of vitamin C in the date flesh and pits (0.179% and 0.137%, respectively) may also play a role in hepatoprotection. Previous in vivo studies indicate that hepatic microsomal drug metabolism decreases in ascorbic acid deficiency and is augmented when high supplements of the vitamin are given to guinea pigs.<sup>12,13</sup> Liver cytochrome P-450 is significantly reduced in ascorbic acid-deficient guinea pigs.<sup>14</sup>

## CONCLUSIONS

This study clearly demonstrates that extracts of date flesh and date pits are effective

agents in the treatment and prevention of CCl<sub>4</sub>-induced hepatic cytotoxicity. The data suggest that the daily oral consumption of an aqueous extract of the flesh and pits of dates, and as a part of the daily diet ad libitum, was prophylactic to CCl<sub>4</sub> poisoning, achieving about 80% protection with date palm flesh and 70% with pits. A similar percentage of protection was achieved when the aqueous extracts of the flesh and pits were used as a cure against CCl<sub>4</sub> poisoning after toxicity was induced.

Of greater importance to the public is the effect of ingesting normal ad libitum levels of date palm (flesh or/and pits), particularly because it is an inexpensive and effective prophylactic and/or treatment against liver cytotoxicity and a dynamic liver support. This study, along with other research, targets *Phoenix dactylifera* L. as a potentially safe and effective plant that has important medicinal values and benefits.

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