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Original Article

Protective Effects of Extract from Dates (*Phoenix Dactylifera* L.) and Ascorbic Acid on Thioacetamide-Induced Hepatotoxicity in Rats

Mohamed Bastway Ahmed, Nabil Abdel-Salam Hasona*
and Hanan Abdel-Hamid Selemain

Department of Chemistry, Biochemistry Branch, Faculty of Science, Beni-Suef University Egypt.

Abstract

The ameliorative activity of aqueous extract of the flesh of dates (*Phoenix dactylifera L.*) and ascorbic acid on thioacetamide-induced hepatotoxicity was studied in rats. Sixty male rats were divided into six equal groups of 10. Two groups were controls, one treated with thioacetamide and one with only distilled water. Two groups received extract of flesh Phoenix dactylifera and intraperitoneal (IP) thioacetamide (400 mg/kg) either before or after administration of flesh extract. Two groups received ascorbic acid and intraperitoneal (IP) thioacetamide (400 mg/kg b.wt.) either before or after administration of ascorbic acid. Liver damage was assessed by estimation of plasma concentration of bilirubin and enzymes activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), γ glutamyl transferase and alkaline phosphatase and serum alpha fetoprotein and serum total testosterone. Treatment with aqueous extract of date flesh or by ascorbic acid significantly reduced thioacetamide-induced elevation in plasma bilirubin concentration and enzymes. This study suggests that thioacetamide-induced liver damage in rats can be ameliorated by administration of extract of date flesh and ascorbic acid.

Keywords: Hepatoprotective; Hepatotoxicity; Phoenix Dactylifera; Thioacetamide; Ascorbic acid.

Introduction

Liver cirrhosis is a worldwide health problem. Reliable, noninvasive methods for early detection of liver cirrhosis are not available (1).

Thioacetamide (TAA) is an experimental hepatotoxin (2), which is a thono-sulfur-containing compound endowed with liver-damaging and carcinogenic activity. Shortly after administration, TAA undergoes metabolism to acetamide and thioacetamide-S-oxide by the mixed function oxidase system (3). The reactive oxygen species from thioacetamide (TAA) induces rat liver cirrhosis that resembles the human disease, and it can serve as a suitable animal model for studying human liver cirrhosis (4).

Water-soluble vitamin C make up an antioxidant system for mammalian cells. Vitamin C or ascorbic acid, is considered the most important antioxidant in plasma and forms the first line of defense against plasma lipid peroxidation (5). Vitamin C may protect lipids and lipoproteins in cellular membranes against oxidative damage caused by toxic free radicals at early stage. The antioxidant function of vitamin C is related to its reversible oxidation

* Corresponding author:
E-mail: n_hasona2003@yahoo.com

and reduction characteristics. Thus, vitamin C may partially prevent certain types of hepatic cellular damage (6, 7).

The date palm (*Phoenix dactylifera*) belongs to the Aracaceae family and is indigenous to the Arabian Peninsula, the Mediterranean, and North Africa countries, parts of India and hotter parts of the USA. Date palm pollen (DPP) has been identified as a potent allergen source (8), with sensitization rates among respiratory patients ranging from 25% in Saudi Arabia (8) to 18.9% in Spain (9), and with higher incidence among residents of rural than of urban communities (10).

The date (*Phoenix dactylifera* L.) has always played an important role in the economy and social life of the people of arid and semiarid regions of the world (11). Egypt is considered to be one of the date-producing countries. The fruit of the date palm is composed of a fleshy pericarp and seed.

Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B_1 (thiamine), B_2 (riboflavin), nicotinic acid (niacin) and vitamin A (12). Recent studies indicate that the aqueous extracts of dates have potent antioxidant activity (13). The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins (14, 15).

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 to treat or prevent some of the toxic actions of thioacetamide (TAA) on the liver in rats. The latter is a model for acute viral hepatitis (16).

Experimental

White male albino rats (Rattus norvegicus) weighing about 200±40 gm were used as experimental animals in the present investigation. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment to exclude any intercurrent infection. The chosen animals were housed in stainless steel cages at normal atmospheric temperature (25±5 °C) and had a 12 h light-dark cycle (light on at 6.00-18. 00h). Food and water were consumed *ad libitum*.

Thioacetamide

Thioacetamide used as an inducer for liver cirrhosis. It was purchased from Sigma Company (United Kingdom).

Induction of liver cirrhosis by thioacetamide

A single injection (400 mg/kg of TAA dissolved in normal saline) was given intraperitoneally (17).

Date fruit (Phoenix dactylifera) and its dose

Date fruits purchased from local market.

Preparation of date fruit extract

The flesh was manually separated from the pits and soaked in cold dist. Water (1: 3 ratio, weight to volume) and kept for 24 h at a temperature 4 °C (18).

Dose of date fruit extract

4 ml/kg of extract was given daily at morning by oral gaveage for all experimental period (19).

Ascorbic acid and its dose

Ascorbic acid was purchased from Sigma company (United Kingdom).

Ascorbic acid dose

100 mg ascorbic acid /kg body weight of rat given daily at morning by oral gaveage for all experimental period (20).

Animal grouping

Group I (n=10): normal control that was orally and daily administered the equivalent amount of the vehicle (distilled water) for the same period.

Group II (n=10): TAA induced control.

Group III (n=10): (Pretreatment experiment-extract): Given 4 ml of extract / kg body weight of rat for 15 consecutive days and treated with single intraperitoneal (IP) thioacetamide and

then treated for 30 days with extract.

Group IV (n= 10) (post-treatment experiment-extract): given aqueous extract for 30 consecutive days and treated with IP TAA on 1ST day of the treatment period.

Group V (n=10) (Pretreated experiment-ascorbic acid): given ascorbic acid for 15 consecutive days and treated with single intraperitoneal (IP) thioacetamide and then treated for 30days with ascorbic acid.

Group VI (n=10) (Post-treatment experiment-ascorbic acid): given ascorbic acid for 30 consecutive days and treated with IP TAA on 1ST day of the treatment period.

By the end of the experimental periods (4 weeks), rats were scarified under diethyl ether anesthesia at fasting state. The portion of blood samples were collected and allowed to coagulate at room temperature; other portion of blood added to it, EDTA (ethylene diamine tetracetic acid) and centrifuged at 3000 r.p.m. for 30 minutes. The clear, non-haemolysed supernatant sera and plasma were quickly removed divided into four portions for each individual, and stored at -20 °C for subsequent analysis.

Liver tissues were quickly excised, weighed and homogenized in a saline solution (0.9%), centrifuged at 3000 r.p.m. for 15 minute and the supernatant were kept at -20 °C for the assay of biochemical parameters related to oxidative stress.

Biochemical Investigations

Blood samples were centrifuged and serum was obtained for determination of glucose concentration according to the method of (21), using reagent kits purchased from the Diamond Diagnostics.

ALT and AST activities in serum was determined according to the method of Reitman and Frankel (22) using reagent kits purchased from BioMerieux Chemical Company (France).

Bilirubin level in plasma was determined according to the method of Jendrassik *et al*., (23) using the reagent kits purchased from the Diamond Diagnostics (Egypt).

Alkaline phosphatase activity in serum was determined according to the method of Rec. GSCC (DGKC) (24) using the reagent kits purchased from the Diamond Diagnostics (Egypt).

Lactate dehydrogenase activity in serum was determined according to the method of Rec. GSCC (DGKC) (25) using the reagent kits purchased from the Diamond Diagnostics (Egypt).

γ-Glutamyl transferase activity in serum was determined according to the method of IFCC methods for measurement of catalytic concentration of enzymes using reagent kits purchased from linear chemicals, S.L. company (Spain).

The AFP Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbant assay (26). serum alpha fetoprotein level was determined using kit purchased from the Diamond Diagnostics (Egypt).

The serum testosterone level was assayed using Coat-a-Count Radioimmunoassay kits (Active1Testosterone RIA DSL-4000, Diagnostic System Laboratories Inc., Texas, USA). The amount of testosterone was expressed as ng/ml.

The content of reduced GSH was determined by modifying the method of Van Dam *et al*.(27). Liver homogenate and a 5% TCA mixture was pre-incubated for 5 min at 4 °C, and then centrifuged at 8000*g* for 10 min at 4 °C. Aliquots of the homogenate were collected to which 5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) was immediately added and incubated for 5 min at 4 °C. The absorbance was measured at 412 nm, and the concentration of GSH was calculated using the absorbance of 1 M of product with $E_{412} = 13\ 600\ M^{-1}\ cm^{-1}$.

A modification of the thiobarbituric acid method was employed for the estimation of malondialdehyde formed (28). Briefly, after incubation for 60 min at 37 °C, a 1.0-ml aliquot of the liver homogenate was mixed with 1.0 ml 40% trichloroacetic acid (TCA), followed by addition of 1.0 ml 2% thiobarbituric acid. The mixture was boiled for 15 min, cooled in an ice bath for 5 min, and then 1 ml 40% TCA was added to it. After standing for 20 min, the mixture was centrifuged at 800*g* for 20 min, and the absorbance of the supernatant read at 532 nm.

Statistical analysis

Statistical analysis were done using the

Table 1. Effect of date palm extract and ascorbic acid on serum levels of glucose, total bilirubin and direct bilirubin of TAA induced cirrhotic rats.

Groups	Serum glucose (mg/dl)	Serum total bilirubin (mg/dl)	Serum Direct bilirubin (mg/dl)
Group (1)	94.52 ±2.07	0.57 ±0.05	0.31 ±0.04
Group (2)	60.73^{+++} ±2.44	4.49^{+++} ±0.17	2.53^{+++}±0.14
Group (3)	70.26* ±2.04	1.81*** ±0.12	1.12*** ±0.09
Group (4)	65.13 ±2.05	2.24*** ±0.14	1.35*** ±0.12
Group (5)	81.89*** ±1.66	1.16*** ±0.10	0.58*** ±0.07
Group (6)	73.92** ±1.74	1.43*** ±0.10	0.78*** ±0.04

Values significantly different compared to normal: $^{+++}$P<0.001
Values significantly different compared to TAA control: *P< 0.05, **P<0.01 and ***P <0.001.

Statistical Package for the Social Sciences (SPSS for WINDOWS, version 11.0; SPSS Inc, Chicago). Comparative analyses were conducted by using the general linear models procedure (SPSS Inc). Values of P<0.01 were considered statistically highly significant and P<0.001 were considered statistically very highly significant.

Results

The TAA induced rats showed a very highly significant decrease in serum glucose level as compared to normal control rat group and the level of serum glucose after treatment by date palm extract and ascorbic acid increase with significant in post treatment with date palm extract and a non significant in post treatment with ascorbic acid as compared with TAA induced rats. Whereas the protective treatment with date palm extract and ascorbic acid showed a very highly significant increase and a highly significant as compared to thioacetamide control group respectively. Both the level of total and direct bilirubin showed an exhibited enormous decrease after treatment as compared with TAA Induced rat (Table 1).

The cirrhotic rats exhibited a highly significant increase (P<0.01) of serum ALT and AST activity as compared to the normal rats. The oral treatment of cirrhotic rats with date palm extract and ascorbic acid exerted a very highly significant decrease (P<0.001) in serum ALT and AST activity as compared to the cirrhotic rats (Table 2).

The cirrhotic rats exhibited a very highly significant increase (P<0.001) of serum ALP, LDH and γ GT activities as compared to the normal rats. The oral administration of date palm extract and ascorbic acid produced marked improvement of the altered serum ALP, LDH and γ GT activities of the cirrhotic rats (Table 3).

The cirrhotic rats exhibited a very highly significant decrease (P<0.001) of serum testosterone level as compared to the normal rats. The oral treatment of cirrhotic rats with date palm extract and ascorbic acid after TAA induction exerted a highly significant increase (P<0.01) in serum testosterone level as compared to the cirrhotic rats. While, the protective groups showed a very highly significant (P<0.001) increase as compared to cirrhotic control group (Table 4).

On the other hand, the serum alpha-fetoprotein level showed a very highly significant increase (P<0.001) as compared to the normal rats. The oral administration of date palm extract and ascorbic acid produced marked improvement (P<0.001) of the altered serum alpha-fetoprotein level of the cirrhotic rats (Table 4).

Table 2. Effect of date palm extract and ascorbic acid on serum activities of ALT and AST of TAA induced cirrhotic rats.

Groups	Serum ALT activity	Serum AST activity
Group (1)	23.25 ± 1.29	39.81 ± 2.36
Group (2)	78.38^{+++} ± 1.61	117.73^{+++} ± 5.06
Group (3)	48.39*** ± 2.08	62.50*** ± 4.04
Group (4)	51.30*** ± 1.71	68.33*** ± 3.52
Group (5)	40.54*** ± 1.26	53.27*** ± 2.78
Group (6)	43.21*** ± 2.12	55.58*** ± 1.20

Values significantly different compared to normal: $^{+++}$P<0.001
Values significantly different compared to TAA control: ***P <0.001.
Units of ALT and AST activity are u/l.

Table 3. Effect of date palm extract and ascorbic acid on serum activities of ALP, LDH and γ-GT of TAA induced cirrhotic rats.

Groups	Serum AL.P Activity	Serum LDH Activity	Serum γ-GT activity
Group (1)	60.04±2.35	41.37 ±2.08	4.92 ±0.48
Group (2)	125.38^{+++} ±5.55	154.65^{+++} ±4.27	81.26^{+++}±2.02
Group (3)	88.03*** ±2.45	103.34***±5.00	51.87***±2.94
Group (4)	98.53** ±1.92	104.77***±4.32	64.72*** ±2.56
Group (5)	72.37*** ±1.86	62.03*** ±2.28	29.99***±1.74
Group (6)	87.16*** ±1.99	81.22*** ±3.45	39.26***±1.52

Values significantly different compared to normal: $^{+++}$P<0.001
Values significantly different compared to TAA control: **P<0.01 and ***P <0.001.
Units of ALP, LDH and γ-GT activity are u/l.

The TAA intoxicated rats showed a very highly significant (P<0.001) decrease in hepatic GSH content, while exerted a very highly significant (P<0.001) increase in hepatic MDA level as compared to normal control group.

The oral administration of date palm extract and ascorbic acid produced a very marked improvement (P<0.001) of the altered hepatic MDA, and GSH content of the cirrhotic rats (Table 5).

Discussion

The reactive oxygen species from thioacetamide (TAA) induces rat liver cirrhosis that resembles the human disease, and it can serve as a suitable animal model for studying human liver cirrhosis (4).

Toxicity experienced by the liver during thioacetamide poisoning results from the production of a metabolite, thioacetamide *s*-oxide, which is a direct hepatotoxin responsible for change in cell permeability and it inhibits mitochondrial activity followed by cell death (29). It has also been reported that chronic thioacetamide exposure produced cirrhosis in rats (30).

An obvious sign of hepatic injury is leakage of cellular enzyme into plasma (31). When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. Their estimation in the serum is a useful quantitative marker for the extent and type of hepatocellular damage (32).

ALT and AST are the most often used and most specific indicators of hepatic injury and represent markers of hepatocellular necrosis. These liver enzymes catalyze transfer of alpha- amino group aspartate and alanine to the alpha-ketoglutaric acid. Whereas ALT is primarily localized to the liver, AST is present in a wide variety of tissue, including heart, skeletal, kidney, brain, and liver. AST is present in both the mitochondria and cytosol of hepatocytes, but ALT is found only in the cytosol. In an asymptomatic person with isolated elevation of AST or ALT level, diagnostic clues can be garnered from the degree of elevation (33).

TAA caused significant increases in the levels of aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase enzymes and that is in agreement with Túnez *et al*. (34).

On the other hand, our study demonstrated that the treatment with *phoenix dactylifera* extract caused marked ameliorations of transaminase enzymes activity (ALT and AST). Our results

Table 4. Effect of date palm extract and ascorbic acid on serum level of testosterone and alpha-fetoprotein of TAA induced cirrhotic rats

Groups	Serum Testosterone	Serum Alpha-fetoprotein
Group (1)	2.90±0.15	1.65±0.16
Group (2)	1.07^{+++}±0.07	9.54^{+++}±0.37
Group (3)	1.49**±0.11	6.44***±0.32
Group (4)	1.39**±0.11	7.17***±0.23
Group (5)	2.00*** ±0.09	4.46***±0.30
Group (6)	1.76***±0.10	4.60***±0.25

Values significantly different compared to normal: $^{+++}$P<0.001
Values significantly different compared to TAA control: **P< 0.01 and ***P<0.001.
Units of testosterone and Alpha fetoprotein are ng/ml.

Table 5. Effect of date palm extract and ascorbic acid on hepatic level of MDA and GSH of TAA induced cirrhotic rats

Groups	Hepatic MDA	Hepatic GSH
Normal control group	113.48 ±3.62	39.93 ± 1.35
Thioacetamide control group	200.59^{+++}±4.12	12.51^{+++}±0.77
Protective phoenix dactylifera group	149.97***±2.59	34.25*** ±1.62
Post treatment phoenix dactylifera group	166.08***±3.50	26.76*** ± 1.21
Protective ascorbic acid group	149.97***±3.07	29.17*** ± 0.96
Post treatment ascorbic acid group	172.86***±3.02	20.75***± 0.68

Values significantly different compared to normal: $^{+++}$P<0.001
Values significantly different compared to TAA control: ***P<0.001.

are in accordance with Al-Qarawi *et al*. (18) who showed the effect of extracts from dates (*Phoenix dactylifera* L.) on carbon tetrachloride–induced hepatotoxicity in rats.

The mechanism by which the date flesh induces its hepatoprotective activity is not certain. However, it is possible that β-sitosterol, a constituent of *Phoenix dactylifera*, 1 is at least partly responsible for the protective activity against CCl4 hepatotoxicity (35). 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 (36). In addition, the recorded content of vitamin C in the date flesh (0.179%) 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 (37). Liver cytochrome P-450 is significantly reduced in ascorbic acid-deficient guinea pigs (38).

In relation to total and direct bilirubin level and alkaline phosphatase and γ-glutamyl transferase activities the mean decrease we have found when extract is administered is significant. This result coincides with the results of Al-Qarawi *et al*.(18).

The present results have clearly demonstrated the ability of TAA to induce oxidative stress in rat liver, as evidenced by the very highly significant rise of lipid peroxidation product (TBARS) and a very highly significant decline of endogenous antioxidants GSH. These findings are in agreement with other reports by Akbay *et al*.(2) and Balkan *et al*.(39).

Blood glucose concentration is known to depend on the ability of the liver to absorb or produce glucose. The liver performs its glucostatic function owing to its ability to synthesize or degrade glycogen according to the needs of the organism, as well as via gluconeogenesis (40).

The blood sugar level after overnight fasting in cirrhotic patients is believed to decrease only in severe hepatic failure (41). This is confirmed by our data that indicate that glucose levels in cirrhosis decreased.

Testosterone is the principal androgen in men (42). The production of testosterone by the male testes is stimulated by luteinizing hormone (LH), which is produced by the pituitary. Testosterone levels change dramatically during the life cycle of males (43).

Hypogonadism (characterized by low testosterone levels and relative hyperestrogenism, loss of libido, sexual impotence and feminine body features in men) is a common complication of advanced liver cirrhosis (44).

The present study results revealed that serum total testosterone and glucose level showed a significant decrease in cirrhotic rats as compared with the non-cirrhotic ones. Whereas, treatment of cirrhotic rats with phoenix dactylifera extract showed a significant increase in serum total testosterone and serum glucose concentrations as compared with that of cirrhotic control rats.

Cao *et al*. (45). indicated that excessive oxidative stress reduced levels of key enzymatic and non-enzymatic antioxidants in Leydig cells,

and resulted in decline in testosterone secretion.

Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extracts in normal functional status of the liver. This is in agreement with the report by Rajkapoor *et al*. (46).

Alpha-feto-protein (AFP) is the most popular tumor marker for hepatocellular carcinoma (HCC). It is used in diagnosis and follow up of cases by estimating its rise in the serum (47).

The TAA control rats showed a very highly significant increase in the level of alpha fetoprotein in serum as compared to the normal rats. This is in agreement with the results of Tournier *et al*. (48) and Dabeva *et al*. (49) who showed that the level of AFP was significantly increased after CCL_4 injection on albino rats.

Conclusions

This study clearly demonstrates that extract of date flesh and ascorbic acid are effective agents in the treatment and prevention of thioacetamide -induced hepatic cytotoxicity. The data suggest that the daily oral consumption of an aqueous extract of the flesh of dates, and as a part of the daily diet ad libitum, was prophylactic to thioacetamide poisoning.

Of greater importance to the public is the effect of ingesting normal ad libitum levels of date palm, 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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