https://doi.org/10.31925/farmacia.2023.2.10 **ORIGINAL ARTICLE**

PROTECTIVE AND ANTIOXIDANT CAPACITY OF DATE PALM SEEDS (*PHOENIX DACTYLIFERA* L.) ON HEPATOTOXICITY IN RATS

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Abstract

In recent years, the population has preferred using phytopharmaceutical products to reduce the complications involving xenobiotics in various pathologies. This study aimed to evaluate the antitoxic activity of three varieties of *Phoenix dactylifera*L. Seeds *in vivo*, namely Deglet Nour, Mech-Degla, and Ghars. Doses of 200 mg/kg bw/day of their extracts were administered to male Wistar albino rats intoxicated using 50% of carbon tetrachloride (CCl_4). The results were compared to silymarin. After 15 days, rats were sacrificed, blood samples were collected for the serum biochemical parameters analysis, and the liver was removed for the oxidative stress enzymes analysis and histopathological study. Extraction results from Deglet Nour, Mech-Deglea, and Ghars yielded different yields of 3.16%, 4.55% and 2.07%, respectively. However, the Deglet Nour variety had the highest antioxidant power with an IC_{50} of 0.231 compared to the other extracts. The polyphenolic contents range between 189.42 and 246.57 mg GAE/mL. Generally, the studied varieties showed exciting results with their regulatory capacity against the serum enzymes rates of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT) that were disturbed by the CCl_4 treatment in addition to the biochemical parameters such as; total protein, glucose levels, cholesterol, and triglycerides. The examined enzymes for oxidative stress were malondialdehyde (MDA) and glutathione (GSH). Several unusual activities were seen in the rats treated with the different seed extracts as a response to the intoxication generated by the administered xenobiotic. The active compounds in the three varieties of date palm seeds outperformed silymarin in terms of regulating power in some metrics. Histological changes accompanied the metabolic response of treated rats to the various extracts. A remarkable inflammation in the liver tissue was observed in the control group. At the same time, the raw extracts of date seeds have proved their capacity to minimize rashes, and the serum biochemical results were similar to the conducted histological study.

Rezumat

Utilizarea produselor fitofarmaceutice a crescut în ultimii ani, populația alegând această opțiune terapeutică în defavoarea xenobioticelor și a reacțiilor adverse pe care le implică administrarea acestora în diverse patologii. Acest studiu a avut ca scop evaluarea activității antitoxice a trei soiuri de *Phoenix dactylifera L. Seeds in vivo*, și anume *Deglet Nour, Mech-Degla* și *Ghars*. Au fost administrate doze de 200 mg/kg corp/zi din cele 3 extracte la șobolani Wistar Albino masculi intoxicați în prealabil cu tetraclorură de carbon 50% (CCl_4), folosindu-se ca și control silimarina. După 15 zile, șobolanii au fost sacrificați, li s-au recoltat probe de sânge pentru analiza parametrilor biochimici serici, iar ficatul a fost prelevat pentru analiza enzimelor de stres oxidativ și pentru studiul histopatologic. Randamentele de extracție din *Deglet Nour, Mech-Degla* și *Ghars* au fost diferite: 3,16%, 4,55% și, respectiv, 2,07%. Cu toate acestea, soiul *Deglet Nour* a avut cea mai mare putere antioxidantă în comparație cu celelalte extracte. În general, soiurile studiate au prezentat rezultate pozitive în ceea ce privește capacitatea lor de reglare a activității enzimelor serice, care au fost dezechilibrate de tratamentul cu CCl_4. Compușii activi din cele trei varietăți de semințe de curmal au fost superiori silimarinei în ceea ce privește puterea de reglare a unor parametri. Modificările histologice au fost în concordanță cu răspunsul metabolic al șobolanilor tratați cu diferitele extracte. La grupul de control s-a observat o inflamație remarcabilă în țesutul hepatic. În același timp, extractele brute din semințele de curmale și-au dovedit capacitatea de a minimiza erupțiile cutanate, iar rezultatele biochimice serice au fost similare cu studiul histologic efectuat.

Keywords: carbon tetrachloride, *Phoenix dactylifera* L., silymarin, antioxidant effect, antitoxic activity

Introduction

The human organism is constantly engaged in relationships with its environments through a set of exchanges that contributes to maintaining poisonings made by xenobiotics, such as heavy metals (*e.g.*, lead, mercury), pesticides (deltamethrin, chlorpyrifos), and some chemical compounds like carbon tetrachloride (CCl_4) [20]. These xenobiotics have harmful effects on human health and risk to the environment. There are two types of poisoning depending on the duration of exposure. The first is acute toxicity noted in the event of massive absorption of high doses within 24 h. The harmful effects of the toxic products appear from the second hour after their absorption and end 24 to 36 hours later [60]. The second is chronic toxicity which occurs with repeated or continuous administration of toxic elements for long periods.

Various complications can be seen after administering poisonous products [35].

The date palm (*Phoenix dactylifera* L.) is a plant that grows in Asia and Africa's desert and hot semi-arid zones. Moreover, dates can be grown in Australia, some American countries where it was introduced in the 18th century, and Mediterranean Europe [13]. Algeria is a major producer of dates, accounting for 6.75%of the output and 3.27% of exports [4]. In 2011, production was expected to be 755.000 Mt, roughly 49 percent of that being Deglet Nour, a popular type among customers. Moreover, Biskra province ranks first with almost 31%, followed by El Oued (27%) and Ouargla (18%) [4, 6]. Generally, in some localities in Algeria, date palms are grown under monoculture focused on the Deglet Noor. Along and other cultivars such as Mech-Degla and Ghars [6]. Date palm fruits have major socio-economic roles for the populations of these regions for which. It provides fruits of high nutritional value food on the one hand. It constitutes a very appreciable source of income for more than 100.000 families in the South of Algeria, with 9% of agricultural exports on the other hand. In these regions, many by-products, such as vinegar, honey, jam and flour, are found [6, 7].

Many research projects have focused on valorising date kernels because date seed palms may offer potential health benefits for humans. Many literature reports mentioned several uses of date seeds which included: the preparation of porous carbon [45], activated carbon [2, 14], supplement in animal feed [22, 24], and as an ingredient in food products such as chocolate [5] and the traditional use of roasted date nuts for preparation of a decaffeinated drink [22, 52]. We used carbon tetrachloride-induced liver injury in Wistar albino rats to examine the antioxidant activity and potential hepatoprotective impact of date seeds from three Algerian types (Deglet Nour, Mech-Degla, and Ghars).

Materials and Methods

Materials

Chemicals

All chemicals utilized in the biochemical tests were of analytical grade. The chemicals necessary for all biochemical assays were procured from Sigma Chemicals Co. (USA), with the exception of CCl4, which was sourced from Sigma-Aldrich Chemical Co. (USA), and silymarin which was obtained from Laboratories Pierre Fabre (Paris, France).

Plant collection and preparation of date seeds extracts

The date pits were acquired from ITDAS (Technical Institute of Saharan Agricultural Development) located in Ain Ben Naoui, Biskra Province, Algeria, during October 2017. The gathered date seeds were completely cleansed using distilled water, dehydrated in an oven at 30°C, and crushed mechanically. Ten grams of date seeds powder were extracted with 200 mL of hot water for 10 min for the three tested cultivars Deglet Nour, Mesh-Degla and Ghars. The resulting extracts were filtrated through filter paper Whatman No°1 and evaporated under decreased pressure instead of being left to dry in the air. Subsequently, the three portions were stored in a refrigerator at a temperature of 4 ± 1°C for the purpose of evaluating their total phenolic content and conducting a DPPH free radical scavenging assay.

Determination of total polyphenol content

The method of Singleton [50] was used to find the total phenolic contents. In short, 200 µL of the thinned-out samples were introduced to 1 mL of the Folin-Ciocâlteu solution. Following a 4-minute incubation period, 800 µL of concentrated sodium carbonate solution (approximately 20%) was included. Next, following a two-hour incubation period at ambient temperature, the absorbance of the reaction mixture was assessed at 765 nm. A calibration plot was established using gallic acid solution (0 - 500 g/mL) as the benchmark standard through the same procedure.

Antioxidant assay

DPPH free radical scavenging assay

Mansouri [33] delineated the assay for scavenging radicals. A solution of DPPH was made by dissolving 2.4 mg of DPPH in 100 mL of methanol. After that, 25 µL of each extract or a standard solution of ascorbic acid was combined with 975 µL of the DPPH solution. The mixture was agitated vigorously and kept in the dark at room temperature for 30 minutes, following which the absorbance values were gauged at 515 nm. The percentage of DPPH scavenging activity was evaluated utilizing the subsequent equation.

$$\% \text{ DPPH scavenging activity} = ((A_{control} - A_{sample})/A_{control}) \times 100$$

$A_{control}$ refers to the absorbance reading of the reaction mixture used as a control, without any test samples added, while A_{sample} refers to the absorbance reading of the reaction mixture with test samples added. To determine the concentration of the extract that can neutralize 50% of the free DPPH radicals, the IC_{50} values were calculated by plotting the inhibition percentages against the concentration of the samples [51].

FRAP assay

Ferric reducing power is determined using a FRAP assay. A solution of 2,4,6-Tripyridyl-s-Triazine (TPTZ is prepared by adding 0.312 g of TPTZ to 100 mL 40 mM HCl. A $FeCl_3$ solution is prepared by adding 0.54 g in an Erlenmeyer flask to 100 mL of distilled water. To create a sodium acetate buffer, combine 2.46 g of sodium acetate and 3.6 mL of acetic acid in an Erlenmeyer flask. Add distilled water until the volume reaches 100 mL. Adjust the pH to 3.6 using acetic acid (CH COOH). For the FRAP solution, mix 10 parts of 300 mM acetate buffer with a pH of 3.6, 1 part of TPTZ, and 1 part of 20 mM ferric chloride.

Heat the mixture at 30°C for 10 minutes before use. This can be performed in test tubes.

Mix 0.15 mL of either the extracts or standard with 2.85 mL of the FRAP solution at room temperature and in the absence of light. Measure the absorbance at 595 nm [3, 58].

Experimental animals

This study employed male Wistar albino rats (274.34 ± 50 g body weight) acquired from the Pasteur Institute, Algiers, Algeria. The rodents were adapted to the laboratory environment for two weeks and housed in large, hygienic polypropylene enclosures, with unlimited access to food and water, under standardized conditions prior to the experiments. The rats were kept at 24 ± 3°C and a 12-hour light/dark cycle was maintained.

Experimental design

The rats were separated into six experimental groups, with six rats in each group. They were given specific treatments for a period of 15 days as described below: Group 1: the control group that did not receive any treatment; Group 2: received CCl_4 (50% concentration in their drinking water); Group 3, 4 and 5: received CCl_4 (50% concentration in their drinking water) along with extracts of Deglet Nour, Mesh-Degla and Ghars (200 mg/kg bw/day), respectively.

The last group, which was also intoxicated with CCl_4, received treatment with silymarin (200 mg/kg bw/day).

Blood collection and tissue homogenate preparation

After 24 h of the last dose of CCl_4, the rat's blood was collected in dry centrifuge tubes, centrifuged at 3000 rpm for 5 min, and the collected serum was utilized for biochemical testing. After the rats were sacrificed, the liver and kidneys were removed, washed in saline solution, dried, and weighed.

The biochemical parameters of the blood samples were analysed, while the liver samples underwent a histopathological examination to obtain PMS (post mitochondrial supernatant). To obtain PMS for later analysis, 0.5 g of the isolated liver from sacrificed rats was homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing potassium chloride (1.17%) and then centrifuged at 800 rpm for 15 minutes to eliminate nuclear debris. The resulting supernatant was subjected to a further centrifugation at 9600 rpm for 45 minutes at 40ºC to obtain PMS.

Determination of serum biochemical parameters

The automatic analyser (COBAS INTEGR® 400 plus Maurimedis) was used to determine the AST, ALT, ALP, g-GT, cholesterol, triglyceride, total protein levels and glucose.

Determination of liver homogenate parameters

Determination of total protein

The overall protein levels in liver homogenates were assessed using the Coomassie Brilliant Blue G-250 technique, with bovine serum albumin serving as a reference standard [55]. A 0.1 mL sample of liver homogenate was added to a 5 mL volumetric flask, and Coomassie Blue was added to fill the flask. The mixture was then vortexed and left to stand for 5 minutes before measuring the resulting blue colour spectrophotometrically at a wavelength of 595 nm.

Determination of glutathione (GSH) content

The Weckberker and Cory method [55] was used to estimate the GSH assay. The liver samples were homogenized in 1 mL of 0.02 M EDTA, and then the homogenate was deproteinized with 0.25% of sulfo-salicylic acid (SSA). After that, 0.8 mL of the homogenate was mixed with 0.2 mL of a combination, vortexed, and kept in an ice bath for 15 min before centrifugation at 1000 rpm for 5 min. The resulting supernatant (0.5 mL) was mixed with 1 mL of Tris-EDTA (0.02 M, pH 9.6) and 0.025 mL of 0.01 M DTNB, and left at room temperature for 5 min. Finally, the optical density was determined at 412 nm.

Determination of (MDA) content

Yagi's method [57] was used to assay MDA. Liver homogenates (100 µL) were mixed with TBA reagent (400 µL) in sealed glass test tubes, heated in a Marie bath at 100°C for 15 minutes, and then cooled in a cold-water bath for 30 minutes while the tubes were open to release any gases formed during the reaction. After centrifugation at 3000 rpm for 5 minutes, the absorbance of the supernatant was measured at a wavelength of 532 nm. The concentration of the thio-barbituric acid reactive substance (TBARS) was calculated using the molar extinction coefficient of MDA (DO sample = $1.53 \times 105\ m^{-1}cm^{-1}$) and expressed in µmol.

$$\text{MDA (µmol/mg of protein)} = (\text{DO sample}/1.53 \times 105)/\text{mg of protein}).$$

Histopathological evaluation

Newly harvested liver tissues, which had been sliced into 2 mm pieces beforehand, were put into plastic cassettes and preserved in 10% formalin for 24 hours. Next, paraffin sections were created through an automated tissue processor and cut into 2 µm slices with a LEICA® TP1020 rotary microtome. Following this, the cells were dyed with haematoxylin and eosin (H & E), and changes in tissue structure were observed through a ZEISS® Primo star photonic microscope [23].

Statistical analysis

The biochemical parameters' outcomes were presented as the mean ± SD. The statistical analysis of the results was performed using the *t-test*. The lowest level of significance was set at $p < 0.05$. All the computations were executed using Excel (2010) and Minitab (version 18) software.

Results and Discussion

Total polyphenol content

The measurement of the polyphenol amount in the extracts of date seeds was carried out utilizing the regression formula of the calibration curve ($y = 0.0007x - 0.0036$, $r^2 = 0.99$), and indicated in GAE,

which was determined to be 246.57 ± 0.002, 193.71 ± 0.003 and 189.42 ± 0.004 mg/mL for Deglet Nour, Mesh-Degla and Ghars extracts, correspondingly.

Antioxidant assay

DPPH free radical scavenging assay

The method for scavenging the DPPH free radical assesses the capacity of a compound or plant extract to function as an antioxidant. When the plant extracts capable of donating a hydrogen atom are combined with a deep violet-coloured DPPH solution, they reduce the solution and the violet colour disappears [41]. Table I displays the findings for the antioxidant activity.

Table I

Radical scavenging potential of date seeds as determined by DPPH assay

IC_{50} mg/mL	Ghars seeds extract	Deglet Nour seeds extract	Mesh-Degla seeds extract
	0.277 ± 0.001	0.231 ± 0.001	0.296 ± 0.001

FRAP assay

The FRAP test revealed values of 45.8 ± 0.1 µmol/g, 41.8 ± 0.1 µmol/g, 47.2 ± 0.1 µmol /g for the Ghars seeds, Deglet Nour and Mesh-Degla extracts, respectively. These values are the highest among the antioxidant activity tests performed.

Effect of date seed extracts on serum ALT, AST, ALP, and γ-GT

Table II displays the safeguarding impact of date seed extract at a concentration of 1% w/w on the alterations induced by CCl_4 in serum levels of ALT, AST, ALP and -GT. Rats exhibited hepatotoxicity after 24 hours due to the final dose of CCl_4, which was demonstrated by a rise in the activities of serum ALT and AST following CCl_4 administration. However, animals that were pre-treated with the date seed extract showed a significant decline in the activities of serum marker enzymes.

Effect of date seed extracts on serum biochemical parameters

Table III displays the levels of serum biochemical parameters in rats across all groups. The CCl_4 group exhibited higher levels of cholesterol, triglycerides, protein and glucose in comparison to the control group ($p < 0.05$).

Effects of date seed extracts on liver total protein and enzymatic antioxidant levels

Liver damage brought about by CCl4 resulted in notable elevations in hepatic antioxidant enzymes such as glutathione (GSH) and malondialdehyde (MDA), as well as overall liver protein. The outcomes of the therapeutic impact of date pits on the total hepatic protein and enzymatic antioxidant levels in CCl_4-induced liver injury can be seen in Table IV.

Histopathological examination

Examination of the control group's liver sections showed a typical histological structure of classic hepatic lobules with central veins and blood sinusoids (Figure.1). Significant vacuolated hepatocytes were found in liver sections of rats treated with CCl_4 alone.

Table II

Serum enzyme activity in control and different treated groups

Treatment	AST IU/L	ALT IU/L	ALP IU/L	γ-GT IU/L
Control	123 ± 0.1	63 ± 0.1	134 ± 0.1	1.57 ± 0.01
CCl_4	933 ± 0.1	647 ± 0.1	207 ± 0.1	2.07 ± 0.01
Ghars + CCl_4	400 ± 0.1	312 ± 0.1	150 ± 0.1	1.18 ± 0.01
Deglet Nour + CCl_4	529 ± 0.1	402 ± 0.1	348 ± 0.1	1.76 ± 0.01
Mesh Deglet+ CCl_4	483 ± 0.1	385 ± 0.1	215 ± 0.1	1.98 ± 0.01
Silymarin + CCl_4	514 ± 0.1	290 ± 0.1	182 ± 0.1	1.28 ± 0.01

Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), γ-Glutamyl transpeptidase (γ-GT).

Table III

Serum biochemical parameters in control and different treated groups

Treatment	Glucose g/L	Protein UI/L	Triglyceride g/L	Cholesterol g/L
Control	0.646 ± 0.002	0.28 ± 0.001	0.79 ± 0.004	0.65 ± 0.001
CCl_4	0.812 ± 0.002	0.5 ± 0.001	0.42 ± 0.004	0.3 ± 0.001
Ghars + CCl_4	0.666 ± 0.002	0.32 ± 0.001	0.35 ± 0.004	0.29 ± 0.001
Deglet Nour + CCl_4	0.67 ± 0.002	0.48 ± 0.001	0.61 ± 0.004	0.39 ± 0.001
Mesh Deglet+ CCl_4	0.695 ± 0.002	0.47 ± 0.001	0.301 ± 0.004	0.21 ± 0.001
Silymarin + CCl_4	0.6675 ± 0.002	0.21 ± 0.001	0.376 ± 0.004	0.31 ± 0.001

Table IV

Impacts of extracts from date seeds on the levels of enzymatic antioxidants in the liver of rats with acute liver damage induced by CCl_4

Treatment	GSH (µmol/min/ mg protein)	MDA (µmol/min/ mg protein)
Control	1.41 ± 0.01	$5.67 \times 10^{-4} \pm 0.001$
CCl_4	0.34 ± 0.01	$8.3 \times 10^{-4} \pm 0.001$
Ghars + CCl_4	0.6 ± 0.01	$5.85 \times 10^{-4} \pm 0.001$
Deglet Nour + CCl_4	0.41 ± 0.01	$4.88 \times 10^{-4} \pm 0.001$
Mesh Deglet+ CCl_4	0.56 ± 0.01	$5.76 \times 10^{-4} \pm 0.001$
Silymarin + CCl_4	1.36 ± 0.01	$6.0 \times 10^{-4} \pm 0.001$

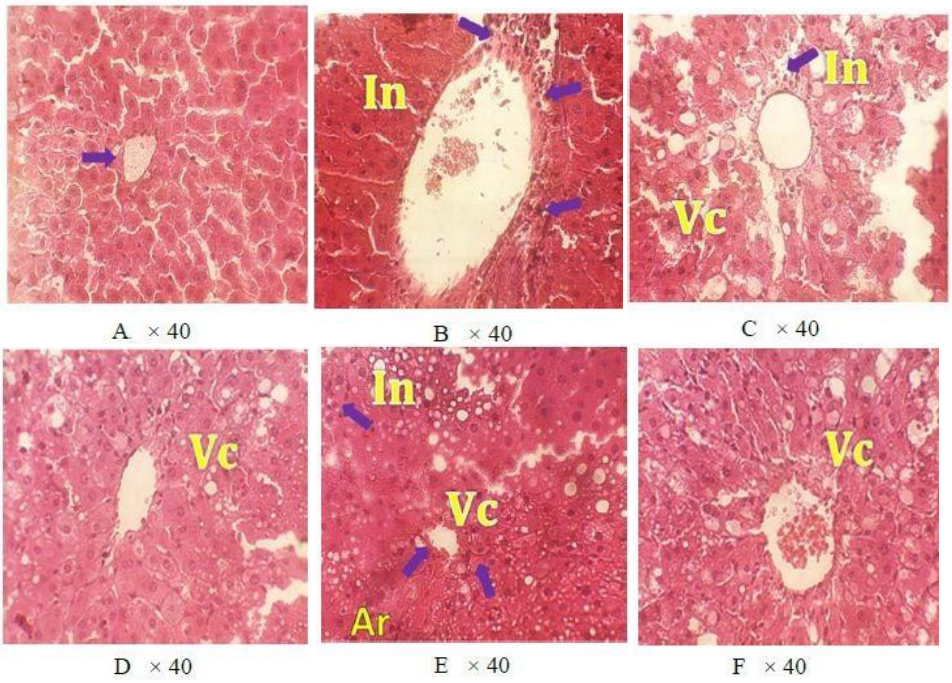

Figure 1.

Effects of *Phoenix dactylifera* seeds and silymarin on histopathological changes induced by the CCl_4 exposure in *Wistar rats.* A**:** control group. B**:** animals treated with CCl_4.C**:** animals treated with CCl_4 and Ghars seeds. D**:** animals treated with CCl_4 and Deglet-Nour seeds. E**:** animals treated with CCl_4 and Mech-Degla seeds. F**:** animals treated with CCl_4 and silymarin

In general, the content of crude extracts varies not only on the varieties of the date kernels of the same family of plants but also depending on the conditions of development and growth of plants (Idaho-thematic conditions), maturity, storage conditions as well as extraction methods [60]. The applied extraction methods play a significant role in extracting (quantitative and qualitative) natural substances in the obtained extracts and indirectly on the biological effects. Water at high temperatures can disrupt cells, facilitating the solvent's penetration and the solubilization of molecules [14]. In our study, the extraction method was the infusion which gave substantial yields of 4.55%, 2.07% and 3.16% of the three varieties of Deglet Nour, Mech-Degla and Ghars, respectively.

The total polyphenols contents of the obtained crude extracts from the three studied varieties, Deglet Nour, Mech-Degla and Ghars, assayed by the Folin-Ciocâlteu method, gave values of the order 246.57 mg GAE/mL, 193.71 mg GAE/mL and 189.42 mg GAE/mL, respectively. In the study of Dalia and Sahar [11] on the nuclei of dates "*Phoenix dactylifera* L." obtained from El-Sharkia (Egypt) using the method that we followed, the obtained results showed an amount equal to 38.8 mg GAE/mL, a value that is considered very low compared to ours. This difference can result from the environmental conditions (Idaho-climatic conditions) on the one hand. On the other hand, the solvents used in the extraction methods play a vital rolein the variation of polyphenol content. Alam Khan [1] published results regarding the antioxidant effect of date nuclei, which showed that acetone, methanol, and ethanol gave high polyphenols amounts compared to water.

The *in vitro* antioxidant effect of the DPPH test, results of the inhibitory concentration of 50% are increased by the show that Deglet Nour extract possessed higher antioxidant activity of 0.231 followed by the Ghars to extract 0.277 and finally, the Mech-

Degla 0.296. The FRAP test confirmed this date seed's antioxidant power and quantified between 45.8 μmol/g and 47.2 μmol/g.

Natural substances *in vitro* antioxidant effect in plant extracts can be attributed to the polyphenols content [38]. A proportional relationship between the polyphenols content of Deglet Nour extract and the *in vitro* antioxidant effect of the same variety [16].

The results of the serum enzymes in CCl4-intoxicated rats were increased compared to the control group. Paradoxically, intoxicated male rats treated with plant extracts from the three date palm varieties revealed a significant reduction in these enzymes. In general, the duration of the experimental protocol and the period of treatment with plant extracts play a critical role in reducing the harmful effects after intoxication [11]. The serum enzymes aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (PAL), and gamma-glutamyl transferase (δ-GT) are four enzymes that are produced in the cytoplasm of cells and are released into the bloodstream when cells are damaged. [40, 49]. These catalysts are considered to be a reliable indication of the destruction of liver tissue and the rejuvenation of liver cells. Therefore, heightened levels of liver catalysts, especially AST and ALT, are often attributed to the metabolic and/or noxious consequences of various substances like psychotropic agents [21], alcohol [30] and pollutants [34], most notably CCl_4 [11]. Rousseau's findings in 1978 [46] confirmed a significant rise in rats exposed to CCl_4 and not treated with serum γ-GT concentrations due to the involvement of epithelial cells in the bile duct. The PAL catalysts are present in the body, especially in the liver, bones, intestines, kidneys, and leukocytes. The involvement of these organs causes the release of PAL [29], so increases in their serum levels reveal an obstruction of the bile ducts [42, 48]. The total protein estimation is one of the standard biological assessments that are part of the complete health assessment, especially for liver and kidney functions. It could also be used to assess nutrition. Protein levels have been disturbed by inflammation induced by CCl_4 [36, 18].

Regarding evaluating lipid parameters (cholesterol and triglycerides), we observed the activity of date palm seed extracts and, precisely, the Deglet Nour variety. This effect can be linked to lipid metabolism activities directly or indirectly. According to Habib study [19], the elevation in lipid levels is related to the enzymatic oxidation by CCl_4 and its transformation into free CCl_3 at plasma membranes. This oxidation leads to the appearance of free radicals or toxic forms of oxygen, which induce lipid peroxidation, destroying cell membranes. Additionally, it has been suggested that CCl_4-induced intoxication leads to a triglyceride catabolism pattern similar to that observed in cases of hepatitis [36]. This situation could also be attributed to reducing lipase activity [25]. The elevation in plasma triglycerides and cholesterol levels can be linked to the alteration of the lipid profile, which is maybe due to a modification in the activity of enzymes that play a role in the metabolism of lipids on the one hand [8]. On the one hand, the content of date nuts in fibre leads to reducing hyperlipidaemia by minimizing the levels of triglycerides and cholesterol [32].

After poisoning with CCl_4, a xenobiotic that produces hyperglycaemia, the glucose level drops. The presence of phenolic compounds in the blood of rats treated with date nucleus crude extract inhibits intestinal glucose uptake and carbohydrate breakdown. These later have suitable inhibitory activities for α-amylase and α-glucosidase, causing an increase in intestinal transit time [1]. Ragunatham and Sulochana [43] claim that phenolic compounds, specifically flavonoids, are responsible for hypoglycaemic activity. Most living organisms possess effective defence mechanisms to prevent and neutralize the damage caused by free radicals. These mechanisms are ensured by a set of endogenous antioxidant enzymes such as MDA, GSH, TBARS, Glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), etc. These enzymes constitute a mutually supportive defence team against reactive oxygen species (ROS) [53].

Based on our study's findings, there is a noteworthy reduction in the blood serum's GSH level, which aligns with the outcomes reported by studies [11, 31, 44]. GSH is one of the most abundant tripeptides widely distributed in hepatocytes. This enzyme displaces free radicals such as H_2O_2 and superoxide anion radicals [15, 39]. Moreover, the GSH constitutes the first line of defence against free radicals; however, this defence process is only partially effective [39]. As GSH is an essential antioxidant in the cell, many enzymes helping its generation. GSH can recover the superoxide anion and the hydroxyl radical by donating its electrons via its thiol group and becoming oxidized [17].

Free radicals can decrease the concentration of intracellular ATP, calcium leakage from the mitochondria, osmotic imbalance and lipid peroxidation by increasing permeability and cell damage [28]. At the same time, natural antioxidants of plant origin can strengthen endogenous antioxidants for defence against ROS and restore the optimal balance by neutralizing them [26]. The study of Manjeshwar [32] reported that the crude extract of date kernels reduced the toxicity of CCl_4 by the increase in the antioxidant plasma "rich in vitamin C, E, A and ß-carotene". These molecules can be responsible for the decrease in lipid peroxidation. The neutralization of free radicals and triplet oxygen [1] and may have anti-cancer properties [32].

MDA is formed when fatty acids are oxidized in the cell membrane and used as a lipid peroxidation indicator. The disturbance of the oxidant/antioxidant balance induced by CCl_4 causes the release of many free radicals, while the natural substances of the

three varieties,Deglet Nour, Mech-Degla and Ghars, may restore this disturbance [10, 54].

In our study, we observed that the use of CCl_4 led to a significant increase in the level of MDA (8.3×10^{-4} µmol/mg protein) in the liver tissues compared to the control group (5.67×10^{-4} µmol/mg protein). On the other hand, treatment with the extracts of the three studied varieties, Deglet Nour, Mech-Degla and Ghars, balanced the lipid peroxidation caused by CCl_4. It reduced the concentration of MDA by 4.88×10^{-4} µmol/mg protein, 5.76×10^{-4} µmol/mg protein and 5.85×10^{-4} µmol/mg protein, respectively. In the same context, the marker for lipid peroxidation decreased in the treated groups with the nuclei of *Phoenix dactylifera* L. to levels close to the control group. These findings are consistent with the study by [19]. They showed that feeding rats with a diet containing 70 mg/kg/day powder of *Phoenix dactylifera* L. seeds significantly reduced MDA in liver tissues. As a result, the seeds of *Phoenix dactylifera* L. that contain anti-oxidant substances have minimized the effect of free radicals.

The variation in the enzymatic levels of GSH and MDA under the action of toxic products, like CCl_4, and their decrease after the administration of the date palm seeds extract highlights the antitoxic properties of natural products of the studied varieties.

Using CCl_4 as a toxic exogenous agent caused histological alterations in the hepatic parenchyma. The xenobiotic-induced local inflammation can become severe in zonal inflammation, which can destroy the vascular walls (either central-lobular or portal veins). The date nuclei have anti-inflammatory properties presented in the treated group with these nuclei [32]. This property of natural substances of the three varieties is very effective depending on the period of intoxication. In another way, natural substances are most effective in the primary stages of intoxication [12, 31]. Studies have presented the toxic effect of CCl_4 in the histology of the liver and the positive impact of using natural products of date nuclei to improve its histological structures [27, 37, 40].

Conclusions

The study evaluated the antitoxic activity of three varieties of *Phoenix dactylifera* L. seeds (Deglet Nour, Mech-Degla and Ghars) in male Wistar albino rats intoxicated with carbon tetrachloride (CCl_4). The extracts showed exciting results in regulating serum enzyme rates and biochemical parameters such as total protein, glucose levels, cholesterol and triglycerides. The extracts also showed excellent activity in handling oxidative stress enzymes (MDA and GSH) and reducing liver inflammation. The Deglet Nour variety had the highest antioxidant power, and the active compounds in the three types of date palm seeds outperformed silymarin in some metrics. Therefore, raw extract from these three date varieties shows promise in combating the toxic effects of CCl_4 and regulating various metabolic parameters.

Conflict of interest

The authors declare no conflict of interest.

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