Effect of Date Palm (Phoenix dactylifera L.) seeds mixed with Burger on CCl₄-induced Hepatotoxicity and Oxidative Stress in Rats

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Nanees Y.E. AWAD*  Rehab Ibrahim Tag El-Deen**

Abstract

Background: Date palm has been shown to have excellent antioxidant activity as a result of containing high flavonoids and phenolic. It has been cited in several studies for the treatment of liver problems, as well as gastric and intestinal disorders. Aim of the study: The present study was administered to inspect the hepatoprotective activity of burgers mixed with date palm seeds on carbon tetrachloride (CCl4)-induced Hepatotoxicity in rats. Materials and Methods: Thirty-six rats were divided into two main groups, the first group (n= 6 rats) was G (1): control (-ve), rats of second main group (n= 30 rats) were injected CCl4 twice a week for two weeks to induce hepatotoxication. After that, divided into 5 groups (each 6 rats) as follow: G (2): Hepatotoxicated rats as a control (+ve) and fed on basal diet only. G (3): Injected rats by CCl4 were fed on basal diet with burgers control, G (4, 5 and 6): Injected rats by CCl4 were fed on basal diet with burgers mixed with date palm seeds (10, 20 and 30g /kg), respectively. Results: Results demonstrated that burgers mixed with date seeds revealed significant improvement blood indicators of CCl4-induced hepatotoxicated rats, hemoglobin (HB) and packed cell volume (PCV), (AST, ALT and ALP) liver function parameters. Also, both serum creatinine and uric acid levels were significantly decreased and nitric oxide (NO). In addition, it restored the antioxidant enzymes activities (glutathione S-transferase (GST), Catalase (CAT) and superoxide dismutase (SOD) that were deficient after

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CCI4 induction. Conclusions: The Date Palm (Phoenix dactylifera L.) seeds DM might be a promising alternative for CCl4-induced liver intoxication protection, and this hepatoprotective activity could be linked to antioxidant activities. It can be utilized as a natural preservative additive in suggestive formulations as a functional food and dietary supplement through biotechnological processes.

Key words: Phoenix dactylifera L., polyphenols, antioxidant, CCL4 and Hepatotoxicity

INTRODUCTION

The Liver is vital organ responsible for metabolic processes. Hepatic damage is correlated to dysfunction of these metabolic processes. Liver dysfunction occurs mainly because of extreme consuming of alcohol, viral infections, xenobiotic exposure and medicine adverse effects or medication interactions. The treatment of liver diseases is still a contentious discussed topic (Kumar et al., 2011 and Kokhdan et al., 2017). Moreover, hepatotoxicity caused by drug-induced is common in developed countries, including Egypt, where people suffer from a variety of liver disorders (Omar et al., 2013). Drug induced liver damage is not preventable during along utilizing drugs in management of chronic diseases which is considered an absolute necessity, for example analgesic anti-inflammatories and anticonvulsants (Hong, 2019). Consequently, increasing liver cell restoration through immune enhancement and avoiding the chain oxidation process as a contributor to liver injury is critical. The concept of employing natural products as antioxidant supplements is based on the synergistic effect of several different types of antioxidant components found in closely related plants (Abdelaziz and Ali, 2014 and Koumbe, 2017).

Liver damage induced by CCl4 (Carbon tetrachloride) is one of the most often used animal models for investigation of hepatoprotective efficiency of numerous substances (Cabre et al., (2000); Kokhdan et al., 2017; Erdemli et al., 2018 and Abu et al., 2021). It stimulated hepatic injury through generating reactive oxidative stress by inducing reactive oxidative stress via biological activation of the cytochrome P-450 system,
which produces the toxic reactive trichloromethyl peroxyl radical, this radical new attack on membrane lipids sets off a chain reaction results in membrane lipid peroxidation, which causes hepatocellular damage and carcinoma (Weber et al., 2003; Basu, 2003; Li et al., 2015 and Abu et al., 2021).

Date palm seeds (DM) are a by-product that comes out in a great amount during the date’s manufacture process (Al Farsi et al., 2005). Date fruits represent approximately 15% of the total weight of dates (Hussein et al., 1998), it is a good source of high nutritive value food as a result of its high content of fiber, minerals, vitamins, lipids and protein. The phytochemical analysis of date palm showed the presence of qualitative and quantities of total polyphenolic compounds and flavonoids such as tannins and anthraquinone glycoside, moreover, it has total phenol content higher than in the edible flesh. Therefore, it is believed that it has the ability to restore liver cells and protect it due to containing high amounts of polyphenolic compounds and flavonoids (Al Farsi and Lee, 2008; Habib and Ibrahim, 2009; Habib et al., 2014A; Bentrad et al., 2017; Sundar et al., 2017; Metoui et al., 2019 and Chinelo et al., 2019). Conventionally, it used in traditional medicine as herbal medication because of its antidiabetic, antirheumatic, choleric, diuretic properties, gastrointestinal complaints, tumors and inflammation (Al-Showiman, 1990; Gogt, 2000 and Schütz et al., 2006). Recently, literatures have revealed that date can act as antioxidants, anti-cholesterol, anti-inflammatory, immune-stimulant, antihyperglycaemic and renoprotective effects (Saryono et al., 2018 and 2019 and Alghamdi et al., 2020).

Hence the date palm seeds (Phoenix dactylifera L.) (DM) are a substantial supply of biologically active constituents could be utilized as a component in meat products; Thus, burgers were mixed with dried date seeds powder (0, 10, 20 and 30g/kg). This study aims to determine chemical composition, phytochemical constituents of (DM), preserve burgers with DM and finally evaluate the protective ability of burgers mixed with palm date seeds against CCl4-induced hepatotoxicity in experimental rats.
MATERIALS AND METHODS

MATERIALS

- Date palm seeds (*Phoenix dactylifera* L.) (DM) were collected as a by-product from Vitrac Food and Drink Company, Cairo, Egypt. Afterwards, DM was washed using tap water, then dried at (50 °C) for a week in the oven. Afterwards, DM were crushed utilizing a commercial blender. It was ground and sieved until it became a fine powder.
- The beef meat was collected from local market Cairo, Egypt.
- Carbon tetrachloride (CCl4), as a toxic chemical substance for liver poisoning, was purchased from El-Gomhouria Company for Trading Grugs, Chemicals and Medical Requirements, Cairo, Egypt (*Passmore and Eastwood, 1986*).
- Paraffin oil, for dilution during the induction, was purchased from a pharmacy in the local market.

METHODS:

**Preparation of Beef Burger Formula:**

Beef Burger blend were prepared as describe by (*Abd-Elhak et al., 2014*) as follows: contained: 71.5, 7.0, 5.0, 5.0, 10.0, of and (g/100g mixed burger) of fat, fine ground onion, bread crust powder, whole egg, rehydrated soy and salt, respectively. The mentioned constituents were minced and divided into four equal quantities, as follows

- **The first sample:** control group (just the mixture of burger without adds).
- **The second sample:** mixed with DM (10 g/kg burger).
- **The third sample:** mixed with DM (20 g/kg burger).
- **The fourth sample:** mixed with DM (30 g/kg burger).

**Determination of chemical composition:**

Moisture content, crude protein, crude fiber, crude fat and ash of DM were determined according to (*AOAC 2007*). Carbohydrates were also calculated by difference.
Determination of total phenolic compounds:

The phenolic compounds of DM were determined in DM ethanolic extract by HPLC according to Goupy et al., (1999) using HPLC Hewllet Packered (series 1050) equipped.

Preparation of beef burger for microbiological analysis:

About 50g of beef burger were aseptically weighed and grinded in sterilized hun. One gram of the grinded Beef Burger was transferred into another sterilized mortar for microbiological analysis where nine ml of sterilized saline solution was added and thoroughly mixed with the beef burgers and this represents 10 dilutions which were then used making further dilution according to Karpinska et al., (2001).

Determination of total aerobic bacteria count:

The aerobic and anaerobic plate count were determined following the procedure proposed by the International Commission on Microbiological Specifications for Foods (ICMSF, 1987). This medium was obtained from El-Gomhouria Pharmaceutical Company Ameriea, Cairo, Egypt. Microbiological examination was carried out every seven days interval from storage at refrigerator temperature. All count were done in triplicates. At each sampling time, the stored bags were analyzed microbiologically according to the procedure recommended by (ICMSF, 1987). Also, Serial dilutions were prepared as described by (ICMSF, 1987).

Experimental animals and diet:

Thirty-six rats weighing an average of (165 ±5g) were obtained from The Laboratory Animals of Helwan Farm. The animals were observed for five days prior to the experiment and fed a standard diet and water ad libitum. The standard diet was performed according to NRC, (1995). The ethical conduct for use and care of animals in this research had been approved by the Research Ethics Committee.

Experimental design:

The experiment was performed in Animal House in the Institute of pathology, Giza. Directly after adaptation, rats were divided into two main
groups: The first group consisted of (n= 6 rats) was fed on the basal diet only and considered as negative control (C-ve) or normal rats. The main second group of rats consisted of (n= 30 rats) in which every rat was injected by 0.5 ml CCl4 diluted by paraffin oil 50 % (3 ml/kg of body weight), and to induce hepato-intoxicition, subcutaneous injection was administered by back twice a week in period of two weeks, according to Jayasekhar et al., (1997). Then, blood samples were collected by the method of hepatic portal vein to liver injury be ensured and liver functions be estimated. After that the rats were divided into 5 groups (6 rats each) beside the first group as follow:

- **Group (1):** The normal rats as a negative control (C -ve group) which feed on basal diet.
- **Group (2):** The hepato-intoxicated rats which kept without any treatment and fed on basal diet.
- **Group (3):** Injected rats by CCl4 then fed on basal diet with burgers control 10g
- **Group (4):** Injected rats by CCl4 then fed on basal diet with DM burgers 10g (10 g /kg).
- **Group (5):** Injected rats by CCl4 then fed on basal diet with DM burgers 10g (20 g /kg).
- **Group (6):** Injected rats by CCl4 then fed on basal diet with DM burgers 10g (30 g /kg).

**Blood sampling:**

At the end of the experiment, all the rats’ groups were sacrificed and blood samples were collected. Blood mixed with heparin was analyzed for (HB) hemoglobin and (PCV) packed cell volume determination according to Drabkin (1949) and Mc Inory, (1954), respectively.

According to Reitman and Frankel (1957); Kind and King (1954); Hare (1950) and Fossati et al., (1980) serum alanine transaminase (ALT), (AST) aspartate aminotransferase, alkaline phosphates enzymes (AP), creatinine and uric acid were determined, respectively.
According to Habig (1974), Claiborne (1985); Beuchamp and Fridovich, (1971) and Green et al., (1981), respectively, (GST) Plasma glutathione transferase, catalase, (SOD) superoxide dismutase enzymes and (NO) nitric oxide were determined.

**Statistical analysis:**

As reported by Snedecor and Cochran (1967), Dunnet’s t-test were used for the analyzation of Differences between groups followed by significant indication analysis of variance (ANOVA) between different groups.

**RESULTS AND DISCUSSION**

**Chemical composition of date palm seeds and burgers mixed with DM**

The proximate chemical composition of DM and burgers mixed with DM (0, 10, 20 and 30g/kg burger) are presented in Table 1. The moisture, fat, protein, ash and carbohydrates % content of date DM were 3.44, 1.17, 5.98, 1.79 g and 87.62, respectively. It could be observed that the addition of palm to burgers result in increment of moisture, fat, protein and ash content when compared to the control burger. Such values are kind of consistent with those mentioned by Al-farsi et al., (2007) who estimated the Omani varieties date palm’ chemical composition as 3.1–7.1%, 2.3–6.4%, 5.0–13.2%, 0.9–1.8% and 22.5–80.2% of moisture, protein, fat, ash and dietary fiber, respectively. However, the chemical composition of date seeds was reported as 16.06 - 33.61%, 5.23-7.02%, 4.88-7.81% and 73.83-82.3% of Moisture, protein, fat and Dietary fiber, respectively (Metoui et al., 2019).

**Table (1): Chemical composition of date palm seeds and burgers mixed with DM**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Moisture %</th>
<th>Fat</th>
<th>Protein</th>
<th>Ash</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date palm</td>
<td>3.44</td>
<td>1.17</td>
<td>5.98</td>
<td>1.79</td>
<td>87.62</td>
</tr>
<tr>
<td>burger control 0% DM</td>
<td>11.50</td>
<td>10.08</td>
<td>22.71</td>
<td>1.45</td>
<td>54.26</td>
</tr>
<tr>
<td>burgers with 10%DM</td>
<td>19.43</td>
<td>12.47</td>
<td>27.77</td>
<td>2.02</td>
<td>38.31</td>
</tr>
<tr>
<td>burgers with 20%DM</td>
<td>17.89</td>
<td>16.60</td>
<td>39.25</td>
<td>2.76</td>
<td>23.50</td>
</tr>
<tr>
<td>burgers with 30%DM</td>
<td>12.89</td>
<td>20.60</td>
<td>34.25</td>
<td>2.98</td>
<td>29.28</td>
</tr>
</tbody>
</table>
**Phytochemical constituents of date palm seeds ethanolic extract**

DM ethanolic extract showed the presence of various qualitative and quantities of polyphenolic compound table (2). The preliminary phytochemical investigation stated that it included significant amounts of vanillic, catechol, pyrogallol, protocatechuic, epicatechein, e-vanillic, p-oh-benzoic, gallic, ellagic, salicylic, chlorogenic, catechin and caffeine, respectively. These results were consistent with Abdelaziz and Ali (2014) who reported that (*Phoenix dactylifera L.*) had high quantities of phenolic compounds (38.8 mg gallic acid equivalent g⁻¹) and total flavonoids (87.86 mg rutin equivalent/g). Also, several reports stated that, date palm DM have large variables of antioxidants (phenolics and flavonoids) (Al-Farsi et al., 2007; Al-Farsi and Lee, 2008 and Juhaimi et al., 2012). Al-Farsi et al., (2007) and Habib et al. (2014 B) revealed that date palm is a rich source of polyphenolic compounds more than grapes, flaxseed, nut DM in comparison and even date fruit; thus, DM could be beneficial in functional food prospects. The flavan-3-ols representing the main class in almost percentage 99% of total polyphenolic compounds and were distributed as epicatechin and catechin. According to Ardekani et al. (2010) it could be utilized in medicinal as well as commercial fields for benefits as containing high amounts of antioxidant (37.42 mmol FeII /100 g dry plant) and (3541 mg gallic acid/100 g dry plant) as aphenolic content. Gallic acid, m-coumaric acid and p-hydroxybenzoic acid have been identified in date DM as same as vanillic acid protocatechuic acid and p-coumaric acid, besides ferulic acid, caffeic acid and o-coumaric acid (Al-Farsi and Lee, 2008). Bijami et al., (2020) identified six compounds which were gallic acid, chlorogenic acid, p-coumaric acid, sinapic acid, catechin, and vanillin as noticed and enumerated by HPLC during analysis of palm DM.
Table (2): Phytochemical constituents of date palm seeds extract

<table>
<thead>
<tr>
<th>phenolic compound</th>
<th>date palm extract ( ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic</td>
<td>357.31</td>
</tr>
<tr>
<td>Gallic</td>
<td>704.34</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>3082.66</td>
</tr>
<tr>
<td>4-Amino-benzoic</td>
<td>28.30</td>
</tr>
<tr>
<td>Protocatchchuic</td>
<td>184.88</td>
</tr>
<tr>
<td>Elagic</td>
<td>539.51</td>
</tr>
<tr>
<td>Catechein</td>
<td>311.05</td>
</tr>
<tr>
<td>Catechol</td>
<td>10928.52</td>
</tr>
<tr>
<td>Epicatechein</td>
<td>1742.94</td>
</tr>
<tr>
<td>Caffeine</td>
<td>264.72</td>
</tr>
<tr>
<td>p-oh-benzoic</td>
<td>967.56</td>
</tr>
<tr>
<td>Caffeic</td>
<td>145.72</td>
</tr>
<tr>
<td>Vanillic</td>
<td>12819.49</td>
</tr>
<tr>
<td>Ferulic</td>
<td>126.40</td>
</tr>
<tr>
<td>Iso-ferulic</td>
<td>64.38</td>
</tr>
<tr>
<td>e-vanillic</td>
<td>1459.53</td>
</tr>
<tr>
<td>Benzoic</td>
<td>152.60</td>
</tr>
<tr>
<td>Salicylic</td>
<td>393.95</td>
</tr>
<tr>
<td>3,4,5Methoxy cinnamic</td>
<td>9.01</td>
</tr>
<tr>
<td>Coumarin</td>
<td>56.95</td>
</tr>
<tr>
<td>p- coumaric</td>
<td>102.18</td>
</tr>
<tr>
<td>Cinnamic</td>
<td>1.77</td>
</tr>
</tbody>
</table>

**Determination of total aerobic bacteria count during storage period of burgers mixed with DM**

The antibacterial (preserving) properties of studied burgers mixed with DM (0, 10, 20 and 30g/kg) against total aerobic bacteria is presented in Table 3. The data showed that there was high influence on the burgers antibacterial activity caused by DM quantities and storage period as it
increased by increasing the amount of DM powder and storage period. This was confirmed by the count of total aerobic bacteria.

The represented results are consistent with Metoui et al., (2019) and Radfar et al., (2020) who stated that DM have antimicrobial properties, especially against Gram-positive bacteria. Radfar et al., (2019) revealed that DM extract included inhibitory effects against Staphylococcus aurous. The antibacterial efficiency of date extracts could be attributed to the presence of phenolic compounds and polyphenols during the protein precipitation process, as well as an inhibitory effect on microorganism enzymes (Metoui et al., 2019). Recently, there were high increment in utilizing natural additive preservatives particularly from plant sources, as a result of the undesirable consequences of utilizing chemical preservative additives in the production of preserved foods (Mir et al., 2018).

Table (3): Determination of total aerobic bacteria count during burger storage period

<table>
<thead>
<tr>
<th>Storage period</th>
<th>Zero time</th>
<th>7 days</th>
<th>15 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control burger</td>
<td>4×10⁶ a</td>
<td>7.3×10⁶ c</td>
<td>13.6×10⁶ c</td>
<td>15×10⁶ c</td>
</tr>
<tr>
<td>Burger preserved using DM (10 g/kg)</td>
<td>1×10⁶ b</td>
<td>1.6×10⁶ ab</td>
<td>1.3×10⁶ a</td>
<td>9×10⁵ ab</td>
</tr>
<tr>
<td>Burger preserved using DM (20 g/kg)</td>
<td>2×10⁶ b</td>
<td>1×10⁶ a</td>
<td>3×10⁵ b</td>
<td>6×10⁵ a</td>
</tr>
<tr>
<td>Burger preserved using DM (30 g/kg)</td>
<td>2×10⁶ b</td>
<td>1×10⁶ a</td>
<td>1×10⁵ a</td>
<td>1×10⁶ b</td>
</tr>
</tbody>
</table>

Each value represents the mean±SD. Means in the same raw with different superscript letters were significant different at P≤0.05.

Effect of burgers mixed with date palm seeds on body weight, body weight gain, food intake and feed efficiency ratio (FER)

The body weight gain, food intake and feed efficiency ratio (FER) of all studied groups are shown in Table (4). Growth indicator of the experimental groups was performed according to the body weight gain just as the experiment ended. Administration of CCl4 for two weeks reduced the weight gain significantly (54.30 g) in (C+ve) group in comparison with the (C-ve) group (85.92g). The rats of group (3) injected by CCl4 then fed with the basal diet with burgers control gained more weight as compared to CCl4
group but fewer than the control group. However, rats of groups (4, 5 and 6) injected by CCl4 and then with the basal diet and burgers mixed with DM (10, 20 and 30 g/kg, respectively) markedly improved the growth performance. Regarding food intake, it could be noted that there were no significant changes between all treatment as compared to negative control. Meanwhile, feed efficiency ratio (FER) for positive control (G2) significantly decreased compared to control group and all treatment groups. This comes in accordance with Abdelaziz and Ali (2014) who reported that (Phoenix dactylifera L.) DM caused weight gain significantly increased in comparison with CCl4 group. On the contrary, non-significant differences in rats’ body weight revealed in-between the experimental (normal, CCl4 treated only and pre-treated with plant extracts then after-treated with CCl4) groups (Thanebal et al., 2021).

Table (4): Effect of burgers mixed with date palm seeds on body weight, body weight gain, food intake and feed efficiency ratio (FER)

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Negative control</th>
<th>Positive control</th>
<th>Control burger</th>
<th>Burger using DM10g/kg</th>
<th>burger using DM20g/kg</th>
<th>burger using DM30g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Weight</td>
<td>115.55 ± 3.17a</td>
<td>110.41 ± 2.50 a</td>
<td>113.14 ± 3.45 a</td>
<td>112.33 ± 2.99 a</td>
<td>110.22 ± 3.11 a</td>
<td>110.34 ± 3.14 a</td>
</tr>
<tr>
<td>Food Intake (g/d)</td>
<td>16.15 ± 2.11 a</td>
<td>14.58 ± 2.17 a</td>
<td>16.12 ± 2.11 a</td>
<td>16.25 ± 2.91 a</td>
<td>16.65 ± 2.18 a</td>
<td>16.25 ± 2.81 a</td>
</tr>
<tr>
<td>Final Weight (g)</td>
<td>200.47 ± 10.11 a</td>
<td>155.71 ± 15.6b**</td>
<td>174.71 ± 10.9 a</td>
<td>190.3 ± 13.8 a</td>
<td>205.5 ± 12.00 a</td>
<td>203.44 ± 15.8 a</td>
</tr>
<tr>
<td>Weight Gain (g)</td>
<td>85.92 ± 11.33 a</td>
<td>54.30 ± 7.71 b**</td>
<td>75.57 ± 8.17 a</td>
<td>88.08 ± 10.22 a</td>
<td>95.92 ± 11.11 a</td>
<td>93.77 ± 11.21 a</td>
</tr>
<tr>
<td>FER</td>
<td>0.093 ± 0.001 a</td>
<td>0.051 ± 0.002 b**</td>
<td>0.084 ± 0.003 a</td>
<td>0.092 ± 0.001 a</td>
<td>0.091 ± 0.001 a</td>
<td>0.094 ± 0.003 a</td>
</tr>
</tbody>
</table>

Each value represents the mean±SD. Means in the same raw with different superscript letters were significant different at P≤0.05.
Effect of burgers mixed with date palm seeds on blood hemoglobin (HB) and packed cell volume (PCV) on rats intoxicated by CCl4

From the obtained results, it could be observed that rats fed on control burgers and burgers mixed DM produced significant increase in (HB) values and (PCV) compared to positive control group (table 5). Meanwhile, non-significant changes revealed in-between control burgers and burgers mixed with DM treatment groups and negative control group. These results were in agreement with Orabi and Shawky (2014) who indicated that date caused significant increase in HG concentration, meanwhile, PCV%, WBCS% and RBCs% didn’t record significant differences in-between the groups date and control which received basal diet.

**Table (5): Effect of burgers mixed with date palm seeds on blood hemoglobin (HB) and packed cell volume (PCV)**

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Negative control</th>
<th>Positive control</th>
<th>Control burger</th>
<th>Burger using DM10g/kg</th>
<th>burger using DM20g/kg</th>
<th>burger using DM30g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB (gm/dl)</td>
<td>13.39 ± 1.18 a</td>
<td>6.19 ± 0.39 b**</td>
<td>11.54 ± 1.4 a</td>
<td>10.95 ± 1.98 a</td>
<td>12.18 ± 2.01 a</td>
<td>12.54 ± 1.82 a</td>
</tr>
<tr>
<td>PCV %</td>
<td>40.01 ± 1.35 a</td>
<td>29.91 ± 3.555 b*</td>
<td>34.79 ± 3.47 b*</td>
<td>35.24 ± 4.01 a b</td>
<td>36.61 ± 4.11 a</td>
<td>36.91 ± 3.17 a</td>
</tr>
</tbody>
</table>

Each value represents the mean±SD. Means in the same raw with different superscript letters were significant different at P≤0.05.

Effect of burgers mixed with date palm seeds treatment on liver and kidney functions

Liver and kidney parameters investigated in this research are represented in Table 6. It could be noted that induction with CCl4 for 2 weeks generated abnormal liver indicators as revealed by increment of serum values of hepatic enzymes AST, ALT and ALP. As well as, when compared to the control group, serum creatinine and uric acid levels increased significantly. However, administration of burgers mixed with DM retrieved normal levels of ALT, AST and ALP as compared to the CCl4
group, while caused a significant decrease in serum creatinine and uric acid levels in comparing with the same group. Additionally, the maximum enhancement of liver and kidney functions accomplished in group (6). Reduction of CCl4-induced in animals treated with burgers mixed with DM enhanced the activities of AST, ALT and ALP levels showing their impact on restoring normal liver functions that been poisoned, and protecting against consequences of CCl4 hepatotoxicity.

There are firm evidences in scientific research which indicated that CCl4 conduction induces hepatic injury experimented on animals revealed increment of serum levels of ALT, AST and ALP (Al-Qarawi et al., 2004; Abdelaziz and Ali, 2014; Liu et al., 2018; Rizk et al., 2020; Abu et al., 2021 and Thanebal et al., 2021). Abdelaziz and Ali (2014) stated that using either the raw (Phoenix dactylifera L. DM) or the roasted significantly reduced the CCl4-induced increments of liver function indicators in serum blood (GOT, GPT and ALP). Moreover, Ahmed et al., (2015) showed that CCl4 induced mice revealed an increase in free radicals leading to kidney and liver injury. On the other hand, giving DM extract reduced levels of ALT, AST, ALP and creatinine and increased the body's antioxidant ability (Al meqbaali et al., 2017). The reducing levels of these indicators as compared to the normal control values is evident on the stability of plasma membranes besides the liver damage restoration. Kowalska et al., (1990) mentioned that (Phoenix dactylifera L.) date’s high content of flavonoids could be contributing element besides its hepatoprotective efficiency through cytochrome P-450 aromatase inhibition. Khan et al., (2018) stated that in mice suffering from hyperlipidemia, the mice consumed Ajwa extract revealed ALT and AST values were higher as compared to the treated mice with atorvastatin only. Alghamdi et al., (2020) reported that as compared to the ischemia/reperfusion (I/R) injury group, date palm extract resulted in significant reductions in serum creatinine and uric acid levels.
Table (6): Effect of burgers mixed with date palm seeds treatment on liver and kidney functions:

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Negative control G1</th>
<th>Positive control G2</th>
<th>Control burger G3</th>
<th>Burger using DM10g/kg G4</th>
<th>Burger using DM20g/kg G5</th>
<th>Burger using DM30g/kg G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (µ/ml)</td>
<td>46.17± 5.81b</td>
<td>77.39± 9.61a**</td>
<td>48.37± 6.01b</td>
<td>52.14± 8.10 b</td>
<td>49.21± 6.15 b</td>
<td>42.21± 4.13 b</td>
</tr>
<tr>
<td>ALT (µ/ml)</td>
<td>13.35± 1.12b</td>
<td>28.55± 3.35 a**</td>
<td>16.71± 1.81 b</td>
<td>15.28± 2.01 b</td>
<td>15.13± 3.51 b</td>
<td>14.11± 3.65 b</td>
</tr>
<tr>
<td>Alk –Pho (µ/ml)</td>
<td>32.17± 5.66 b</td>
<td>50.38± 5.81 a**</td>
<td>39.80± 4.11 b</td>
<td>37.73± 4.37 b</td>
<td>36.34± 5.01 b</td>
<td>35.11± 3.11 b</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.78± 0.01b</td>
<td>1.96± 0.11 a**</td>
<td>0.98± .02 b</td>
<td>0.88± 0.12 b</td>
<td>0.75± 0.13 b</td>
<td>0.74± 0.15 b</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.83± 0.26c</td>
<td>4.41± 1.01 a***</td>
<td>2.11± 0.81b*</td>
<td>2.52± 0.77 b*</td>
<td>2.29± 0.67 b*</td>
<td>1.95± 0.74 c</td>
</tr>
</tbody>
</table>

Each value represents the mean±SD. Means in the same raw with different superscript letters were significant different at P≤0.05.

Effect of burgers mixed with date palm seeds treatment on antioxidant enzymes levels (glutathione S-transferase (GST), Catalase and superoxide dismutase (SOD) and nitric oxide (NO))

As shown in Table 7, CCL4 administration caused an elevation of nitric oxide (NO) level and a demotion in glutathione S-transferase (GST), Catalase (CAT) and superoxide dismutase (SOD) compared to the control group. However, all groups treated with burgers mixed with DM restored normal levels of GST, Catalase and SOD and caused significant decrease in nitric oxide (NO) levels compared to CCL4 group. These results is agreement with Liu et al., (2018); Rizk et al., (2020); Abu et al., (2021) and Thanebal et al., (2021) established that the administration of CCL4 affected antioxidant enzymes (SOD, CAT and Glutathione peroxidase (GPx)) by decreasing their activities in liver tissue homogenate significantly, in contrast, the oxidative stress parameter Malonyl-di-Aldehyde (MDA) value in the homogenate was significantly increased. Malondialdehyde level (as a reactive substance of thiobarbituric acid) and endogenous antioxidant enzymes activities such as SOD, GPx and CAT are sensitive indices in free radical induced hepatocellular damage (Mohajeri et al., 2011). Moreover, Abu et al., (2021) indicated that a significant decrease
of SOD, GPx and CAT in the hepatic tissues of rats induced by CCl4 may have been caused by high amounts of free radicals produced by CCl4 and possibly developed inactivation or inhibition of the synthetic pathways of these endogenous antioxidant enzymes thereby resulting in their low turnover. Abdelaziz and Ali (2014) and Ahmed et al., (2015) established that rats fed on an experimental diet contained (Phoenix dactylifera L.) DM powder resulted in higher levels of both SOD and GST besides NO level significantly decreased compared to CCl4 group in experimental rats. These indications showed that (Phoenix dactylifera L.) DM have antioxidant compound that controlled the CCl4-induced oxidative stress in liver tissues. Also, these results agree with Paranatham and Ali et al., (2012) who revealed that flavonoid contents of (Phoenix dactylifera L.) DM have antioxidant properties. Phytochemicals such as phenolic acid and flavonoid may have antioxidant properties against the alterations of superoxide and hydroxide free radicals, as well as hydrogen peroxide, restoring the antioxidant status in the cells. (Etim et al., 2008). Alghamdi et al., (2020) stated that pre-treatment utilizing date palm fruit or date palm extracts resulted in significant increase in CAT activity and GSH concentration.

**Table (7): Effect of burgers mixed with date palm seeds treatment on antioxidant enzymes levels (glutathione S-transferase (GST), Catalase and superoxide dismutase (SOD) and nitric oxide (NO)***

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Negative control G1</th>
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<th>Burger using DM20g/kg G5</th>
<th>Burger using DM30g/kg G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST (µ/l)</td>
<td>288.31± 33.27 a</td>
<td>77.85± 8.40 c***</td>
<td>188.35± 22.17 b*</td>
<td>211.31± 23.81 b</td>
<td>240.21± 23.71 a</td>
<td>278.15± 31.71 a</td>
</tr>
<tr>
<td>Catalase (µ/l)</td>
<td>285.21± 55.14 a</td>
<td>102.55± 10.14 c***</td>
<td>230.77± 32.11 ab</td>
<td>291.61± 31.61 a</td>
<td>277.11± 30.91 a</td>
<td>284.11± 39.11 a</td>
</tr>
<tr>
<td>SOD (µ/l)</td>
<td>70.13± 5.22 a</td>
<td>46.25± 3.47 b***</td>
<td>63.14± 7.16 a</td>
<td>68.33± 6.35 a</td>
<td>71.31± 9.23 a</td>
<td>73.14± 7.81 a</td>
</tr>
<tr>
<td>NO (µmol/l)</td>
<td>2.17± 0.33 b</td>
<td>10.11± 1.44 a***</td>
<td>4.33± 1.11 b</td>
<td>3.22± 1.03 b</td>
<td>3.11± 1.05 b</td>
<td>2.01± 1.21 b</td>
</tr>
</tbody>
</table>

Each value represents the mean±SD. Means in the same raw with different superscript letters were significant different at P≤0.05.
Conclusions

In conclusion, the alimentation on burgers mixed and preserved with date palm seeds might be considered as a nutritive source rich in natural fibers and phytochemicals and could be a valuable approach to avoid the hepatotoxicity stimulates by carbon tetrachloride. These protective capabilities could be explained, at least in part, by the high levels of antioxidants compounds (polyphenols and flavonoids). Besides it would be an economically viable alternative to special healthy meat products. Nevertheless, additional research is needed to better understand the valuable components and mechanisms concerned in this protective effect of date palm.

REFERENCES


Effect of Date Palm (Phoenix dactylifera L.) seeds Mixed with Burger on CCl4-induced Hepatotoxicity


Effect of Date Palm (Phoenix dactylifera L.) seeds Mixed with Burger on CCl4-induced Hepatotoxicity


تأثير البرجر المخلوط ببذور ثمر النخيل على سمية الكبد والالتهاب التأكسدي المستحدث برابع كلوريد الکربون في الفئران

الملخص

تم تغذية الفئران بهجوم من البرجر المخلوط ببذور ثمر النخيل والكربون (CCl4). القسمت ستة وثلاثون فأرة إلى مجموعتين رئيسيتين، المجموعة الأولى (ن = 6) كانت الفئران المجموعة الرئيسية الثانية (ن = 30) جرداً تم حقنها برابع كلوريد الکربون مرتين في الأسبوع لمدة أسبوعين تحت نظام التغذية الكبدى. بعد ذلك قسمت إلى 5 مجموعات (كل مجموعة 6 فئران) على النحو التالي: المجموعة (1) الفئران المصاب بالسمية الكبدية وتغذى على الوجبة الأساسية فقط والمجموعة (2) تغذى الفئران المحكونة بواسطة رابع كلوريد الکربون على الوجبة الأساسية مع البرجر المخلوط ببذور ثمر (10، 20، 30 جم / كجم). على التوالي. أظهرت النتائج أن البرجر المخلوط ببذور ثمر أظهر تحسيناً للدورة الدموية (ALT و AST) و PCV مؤشرات الدم في الفئران المصابين بسمية الكبدية. الهيموجلوبيين و ALP. كما أن مستويات الكرياتينين في الدم وحمض البوليك انخفضت معنويًا وأكسيدي (ALP) حيث استعاد نشاط الإنزيمات الضاردة للجسم (CAT، SOD) و (GST) ترتبط بـ CCl4 منخفضة بعد الإصابة المستحثة بـ CCl4 الاستنتاجات: برن تغذية الفئران هل تكون بديل واعد للحماية من السمية الكبدية والالتهاب التأكسدي المستحث بـ CCl4 هذل نشاط وقائي للكبد مرتبط بنشاط المضادات للأكسدة. يمكن استخدامها ضعفاء حفاظة طبيعية لـ التركيبات المشتركة في المفاعلات وظيفي والعلامات الغذائية من خلال العمليات التكنولوجية الحيوية.

الكلمات المفتاحية: بذور ثمر النخيل، الفئران، مضادات الأكسدة، رابع كلوريد الكربون، السمية الكبدية.