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Hepatoprotective activity of date fruit extracts against dichloroacetic acid-induced liver damage in rats

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ABSTRACT

The hepatoprotective activity of an aqueous date extract (ADE) against dichloroacetic acid (DCA) induced liver damage in rats was investigated. The free radical scavenging activity of ADE was evaluated using the DPPH assay. The total carbohydrate phenolic, flavonoid and condensed tannins contents of the ADE were determined. Different polyphenolic compounds, namely gallic, chlorogenic, protocatechuic, ferulic, caffeic, syringic, m-hydroxybenzoic, coumaric and phenylacetic acids, and catechin, were indentified. Oral administration of the ADE to male Wistar intoxicated with DCA at 0.5 and 2 g/l as drinking water for 2 months, demonstrated a significant protective effect by lowering the levels of hepatic marker enzymes (AST, ALT, LDH and GGT) and conjugated bilirubin, and by improving the histological architecture of the rat liver. ADE attenuated oxidative stress by decreasing the extent of hepatic TBARS (thiobarbituric acid reactive substances) formation, restoring the activities of SOD, CAT and GPx and by reducing the hepatic DNA fragmentation. This study demonstrated that ADE protects rat liver from DCA-induced injury and suggests a potential therapeutic use for ADE.

1. Introduction

Evidence from both *in vitro* and *in vivo* studies has linked environmental factors such as radiation, xenobiotics and chlorinated compounds as significant inducers of cellular damage via reactive oxidative species (ROS)-mediated toxicity (Galaris, Skiada, & Barbouti, 2008). Dichloroacetic acid (DCA) is a major by-product of water disinfection by chlorination and is a probable minor metabolite of trichloroethylene and is used as

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fungicide (Bull, Sanchez, Nelson, Larson, & Lansing, 1990; DeAngelo, Daniel, McMillan, Wernsing, & Savage, 1989). DCA is also used for therapeutic purposes, especially for the treatment of lactic acidosis in patients with mitochondrial dysfunction (Mori, Yamagata, Goto, Saito, & Momoi, 2004; Spruijt et al., 2001). However, long term treatment of patients with a certain dose (10–50 mg/kg/day) of DCA was found to be associated with adverse effects that included mild liver dysfunction, hypoglycaemia and changes in the central and peripheral nervous system (Mori et al., 2004; Spruijt et al., 2001).

DCA is rapidly absorbed into the bloodstream from the gastrointestinal tract in rats and mice (Schultz, Merdink, Gonzalez-Leon, & Bull, 2002). It is initially distributed to the liver and muscle, and subsequently to other target organs (James et al., 1998). Several studies have demonstrated that DCA exhibits hepatocarcinogenic effects in rodents when administered in the drinking water (Bull et al., 1990; DeAngelo & Daniel, 1996). Beside the induction of metabolic and pathological changes in the liver including lipid peroxidation (Larson & Bull, 1992), DNA-single strand breaks (SSBs) (Hassoun, Cearfoss, & Spildener, 2010), peroxisome proliferation (DeAngelo et al., 1989), liver hypertrophy and glycogen accumulation (Bull et al., 1990; Mather, Exon, & Koller, 1990).

Liver diseases remain one of the serious health problems and no satisfactory protective drugs are available. For this reason the use of natural treatments from food or medicinal plants is considered to be effective and safe for hepatotoxicity, due mainly to the presence of various antioxidant compounds (flavonoid and phenolic acid compounds, trace elements like selenium, etc.). Date is the fruit of *Phoenix dactylifera* L., and listed in folk medicine for the treatment of various chronic diseases and cancer. Date fruit passes through different stages of ripening; *hababouk* (pea-sized immature fruit), *kimri* (green date with a hard texture and contains the highest moisture and tannin levels), *besser* or *khalal* (coloured stage, fruit still has a firm texture, maximum size and weight and moisture contents up to 50–60%), *rutab* (fruit is less astringent, with a soft texture and darker colour) and *tamr* (whole fruit becomes dark brown in colour, with a soft texture and wrinkled appearance) (Baliga, Baliga, Kandathil, Bhat, & Vayalil, 2011). Dates could be consumed as fresh fruit at *besser* and *rutab* stages (short shelf life), or at *tamr* stage (good storability). The analysis of the phytochemical composition of date fruit during different ripening stages showed that the highest amount of polyphenolic compounds, carotenoids and anthocyanins was found in the earlier stages (*kimri* and *besser*) and decreased as ripening progressed (Boudries, Kefalas, & Hornero-Mendez, 2007; Eid, Al-Awadi, Vauzour, Oruna-Concha, & Spencer, 2013; El Arem et al., 2012b). The *besser* stage was found to be very rich in flavonoids, tannins and phenolic acids such as ferulic, caffeic, *p*-coumaric and protocatechuic acids, and catechin, and had the greatest antioxidant activity as compared with *rutab* and *tamr* stages (Eid et al., 2013; El Arem et al., 2012b).

Several research groups have reported that date fruit extract at *rutab* and *tamr* stages participate in the prevention of hypertension, hyperglycaemia, hepatotoxicity, hypercholesterolaemia and oxidation of lipoproteins, enhancing serum antioxidant status, alleviating the harmful effects of oxidative stress and inflammation on the vascular system (Al-Shoaibi, Al-Mamary, Al-Habor, Al-Zubairi, & Abdelwahab, 2012; Saafi et al., 2011; Vayalil, 2012). The protective effects of date fruit against the previously mentioned diseases are attributed to vitamin C, trace elements, and to bioactive compounds such as carotenoids, sterols, tannins, isoflavones, lignans, flavonoids and other polyphenols especially phenolic acids. These phytochemicals confer inhibitory effects against oxidative damage (Vayalil, 2012). However, no study has investigated the beneficial effect of date fruit or date fruit extract, at *besser* stage, against liver damage induced by xenobiotics.

According to a previous study (Vayalil, 2002), the aqueous extract of date fruit was found to be a strong scavenger of reactive oxygen species like superoxide ($O^{\bullet -}$) and hydroxyl radical ($^{\bullet}OH$) and showed a strong inhibitory effect *in vitro* on macromolecular damages such as lipid peroxidation and protein oxidation. Therefore, the present study was planned to investigate the protective effect of the aqueous extract of Degla cultivar, at *besser* stage, against dichloroacetic acid induced hepatotoxicity in male Wistar rats.

2. Materials and methods

2.1. Plant materials

Degla cultivar was collected from the station of Souk Lahad (Kébili, south of Tunisia) at *besser* stage.

2.2. Total carbohydrate content

Total carbohydrate was determined according to the phenol–sulphuric acid method as described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). The sample (1 ml) was mixed with 1 ml phenol solution (5%, w/v) followed by addition of 5 ml concentrated sulphuric acid. The sample was left at room temperature for 30 min prior to measuring absorbance at 485 nm using a spectrophotometer (Jenway visible spectrophotometer 115 VAC model, Cole-Parmer, Chicago, IL, USA). The total amount of carbohydrate was determined based on a standard calibration curve prepared using glucose.

2.3. Date palm fruit extract preparation

The aqueous date extract (ADE) was prepared according to the method of Al-Qarawi, Mousa, Hamed Ali, Abdel-Rahman, and El-Mougy (2004). The flesh was manually separated from the pits and soaked in distilled water (1:3 ratio, w/v) and kept for 48 h at 4 °C with continuous stirring. The mixture was then centrifuged (Hettich universal 320R centrifuge, Tuttlingen, Germany) at 4 °C for 20 min at 4000 *g* and the supernatant was collected and used immediately in subsequent experiments.

2.4. Phytochemical analysis of the ADE

Total phenolic content (TPC) was estimated using Folin–Ciocalteu reagent as described by Al-Farsi, Alasalvar, Morris, Baron, and Shahidi (2005). Calculations were based on a calibration curve obtained with gallic acid. TPC was expressed as mg of gallic acid equivalents (GAE) per 100 *g* of fresh weight