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Phoenix dactylifera protects against oxidative stress and hepatic injury induced by paracetamol intoxication in rats

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ABSTRACT

The current studies were sought to determine effects of antioxidant potential of aqueous and methanolic extracts of *Phoenix dactylifera* leaves (PLAE and PLME) against the widely-used analgesic paracetamol (PCM) induced hepatotoxicity. Groups of rats were treated with or without PCM (1500 mg/kg), PLAE and PLME (300 mg/kg) and n-acetylcysteine (NAC, 50 mg/kg) followed by assessments of liver function tests, oxidative stress, antioxidant defenses, and hepatotoxicity. We observed that PCM significantly elevated serum liver markers, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), and bilirubin compared to control (untreated) group. These PCM-induced effects were associated with oxidative stress as demonstrated by increased levels of malondialdehyde (MDA) and reduced levels of hepatic antioxidant enzymes, glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Pretreatment of PLME decreased ALT and AST by 78.2% and tissue MDA by 54.1%, and increased hepatic GPx (3.5 folds), CAT (7 folds) and SOD (2.5 folds) compared to PCM group. These PLME-mediated effects were comparable to NAC pretreatment. Histological analysis demonstrates that PLME conserved hepatic tissues against lesions such as inflammation, centrilobular necrosis, and hemorrhages induced by PCM. In contrast, PLAE-mediated effects were less effective in reducing levels of liver function enzymes, oxidative stress, and liver histopathological profiles, and restoring antioxidant defenses against PCM-induced intoxication. These findings indicate that PLME exerts protective effects against PCM-induced hepatotoxicity via scavenging free radicals and restoring hepatic antioxidant enzymes. Thus, PLME and its bioactive components could further be evaluated for their pharmacological properties against drug-induced deleterious effects.

1. Introduction

Reactive oxygen species (ROS) are free radicals which affect various biological and pathophysiological processes including liver ailments, diabetes, heart diseases, cancer, and aging [1]. Molecular redox processes including thiol and NAD/NADP systems inside the cell cause production of superoxide anions ($O_2\cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot^-$) which are components of ROS [2]. These free radicals can have physiologically beneficial effects when produced at low levels, but at high levels, they can lead to oxidative stress and cellular damage [3].The latter effect occurs due to electron pairing of ROS with cellular macromolecules such as lipids, leading to a chain

Abbreviations: AAP, 4-aminophenazone; ALP, alkaline phphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; CYPs, cytochrome P 450s; DHBS, 3,5-Dichloro -2-hydroxybenzene sulfonic acid; EDTA, ethylenediaminetetraacetic acid; GGT, gamma glutamyl transferase; GSH, glutathione; GPx, glutathione peroxidase; H2O2, hydrogen peroxide; MDA, malondialdehyde; NAC, n-acetylcysteine; NAPQI, N-acetyl-p-benzoquinoneimine; NO, nitric oxide; O2·–, superoxide anions; OH·–, hydroxyl radicals; ONOO–, peroxynitrite; PBS, phosphate buffer saline; PCM, paracetamol; PLAE, *Phoenix dactylifera* leaves aqueous extract; PLME, *Phoenix dactylifera* leaves methanolic extract; ROS, Reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid

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reaction of lipid peroxidation, DNA damage and dysfunction of proteins and enzymes, especially containing sulfur moieties [2]. The imbalance between the levels of pro-oxidants and cellular antioxidant defenses including SOD, CAT, and GPx enzymes contribute to an increase in cellular oxidative stress [4].

Paracetamol (PCM) is a widely-used antipyretic and analgesic drug for people of all ages [5,6]. PCM undergoes biotransformation via cytochrome P 450s (CYPs) including CYP2E1, CYP3A4 and CYP1 A2 into a highly reactive radical, N-acetyl-p-benzoquinoneimine (NAPQI), which can be detoxified with glutathione (GSH) conjugation when produced at low levels [7,8]. Accumulating evidences indicate that PCM at large doses can produce high levels of NAPQI that exceed the amount of GSH needed to metabolize it, which can cause enhanced ROS generation, lipid peroxidation, mitochondrial dysfunction, depletion of ATP, DNA breakdown and apoptosis [8]. These events could lead to oxidative damage of hepatic cells (i.e. hepatotoxicity) leading to subsequent elevation of bilirubin and liver enzymes in the serum such as AST and ALT [9]. Supplementation of antioxidants has been shown to attenuate oxidative stress-induced deleterious effects. Notably, n-acetylcysteine (NAC), the SH-containing antioxidant compound serves as a clinical antidote to PCM-induced toxicity [10]. NAC treatment has been shown to exert protective effects against PCM-induced hepatotoxicity via its ability to scavenge free radicals and restore depleted GSH [11]. However, there are limitations on its use due to the associated side effects such as nausea, diarrhea, headache and anaphylactic reactions [12]. In recent years, the synthetic medical compounds being practiced to treat or manage liver diseases have exerted various adverse effects [13]. To that end, natural antioxidants from plant sources have been shown to exert preventive effects against oxidative stress compared to chemical compounds [13,14,15]. As large population in developing countries of Asia and Africa still use traditional medicines for various ailments [16], bioactive compounds of natural resources could offer the safe and effective alternative means to manage drug-induced adverse effects [17].

Among these natural resources, date palm tree (*Phoenix dactylifera* L.*)* is a member of the family Arecaceae [18]. The production of dates in Arab countries constitutes about 80% of the total world production [19], which are considered as one of the most important traditional foods for Arabian people. The different parts of *P. dactylifera* tree are used in traditional medicine in Arab countries, especially in Egypt, as a remedy for diabetes, gastrointestinal problems, liver diseases, pharyngitis, fevers, and venereal diseases like gonorrhea [20]. The phytochemical analysis has revealed its richness in phenolics, carbohydrates, sterols, carotenoids, anthocyanins, procyanidins, flavonoids, vitamins and tannins [18,21,22,23]. Notably, *P. dactylifera* leaves are rich in total polyphenols, flavanoids, and flavonols, which possess potent antioxidant properties [24]. These phytoconstituents properties of *P. dactylifera* leaves have been shown to exert beneficial biological and pharmacological effects including the potent antioxidant [19], hepatoprotective [25], anti-hyperlipidemic [26] and antiviral [27] activities. Importantly, treatment of *P. dactylifera* leaves have been shown to improve the lipid profiles in alloxan-induced diabetes in rats [28]. However, to the best of our knowledge, the in vivo antioxidant, and hepatoprotective potentials of *P. dactylifera* leaves have not been studied. The current study sought to determine effects of methanolic (PLME) and aqueous (PLAE) extracts *of P. dactylifera* leaves against the hepatotoxic effect and oxidative stress induced by PCM in a rat model.

2. Materials and methods

2.1. Chemicals

Paracetamol (PCM) was purchased from Almaya Co. (Libya), and n-acetylcysteine (NAC) from Labortoires Galpharma (Tunisia). Kits for the determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), and total bilirubin were purchased from Biomaghreb Laboratories. The kits for GPx, CAT, SOD, and MDA were purchased from Biodiagnostics Co. (Cairo, Egypt). All other chemicals were purchased from standard commercial suppliers and were of analytical grade. All solutions were prepared immediately before use.

2.2. Preparation of PLAE and PLME

Leaflets of *Phoenix dactylifera* L. Hammory cultivar (family Arecaceae) were collected from different areas in Misurata, Libya. A voucher specimen (ph-d.3) was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Misurata University, Misurata, Libya. These leaflets were cleaned, dried sun and cut into tiny pieces. A watery extract was prepared by boiling 200 g m of date leaflets in 500 ml distilled water then filtering twice. The filtrate was lyophilized using a freeze dryer. For the preparation of the methanolic extract, the small pieces of date leaflets were crushed into powder. After that, 200 g m of this powder was macerated into 700 ml methanol (1:3.5 w/v) for 48 h at room temperature away from the light, then the mixture was filtered three times using filter paper to obtain a clear filtrate. The filtrate was concentrated in a rotary evaporator in a vacuum at 60 °C and dried further at 45 °C. The blackish brown lyophilized watery extract was stored at room temperature away from the air, and the blackish green methanolic extract was stored in a refrigerator. Sterilized distilled water was used as a solvent in order for the two extracts to be administered orally to mice for experimental purposes.

2.3. Preliminary phytochemical analysis

PLME and PLAE were subjected to preliminary phytochemical analysis for the detection of the following compounds; alkaloids, steroids, terpenoids, saponins, tannins and polyphenolic compounds and flavonoids as per the published reports [29,30].

2.4. Acute toxicity study of PLME and PLAE

Thirty five albino mice were used for the acute toxicity study. These mice were divided into 3 groups of 5 animals each to receive PLAE, PLME, and normal saline. These animals were fasted for 3 h before the administration of PLAE and PLME at 100, 300 and 2000 mg/kg doses by oral gavage to determine the safe doses of these extracts. Each individual animal was observed every 30 min for the first 4 h, then every hour for 24 h, then twice a day for the next 2 weeks. The observational parameters included behavioral analysis such as mood and alertness; neurological analysis such as central excitation and inhibition, muscle tone and coordination, reflexes, body temperature, and pain as well as autonomic analysis such as pupil size, heart rate, secretions, excretions, rate and depth of respiration. The mouse number and their time of mortality were recorded. All the procedures were in accordance with Organization for Economic Co-operation and Development (OECD) guideline 423 [31].

2.5. Animal care and monitoring

Male Sprague-Dawley rats of 4–5 months old weighing 250–300 g m, and albino mice of 2-3 months old weighing 25-30 gm were randomly allocated to polypropylene cages having autoclaved wooden shaving beddings maintained under standard controlled conditions of 12 h light/dark cycle with an ambient room temperature of 25 ± 2 °C and relative humidity of 55 ± 5. These animals were fed with commercial pellet diet and water *ad libitum*. All treatments were in accordance with the animal care guidelines of the Institutional Animal Ethics Committee, Misurata University, Faculty of Pharmacy.