Aqueous Extract of Date Palm Fruit (*Phoenix dactylifera*) Protect Liver Against Cyproterone Acetate Toxicity in Male Mice

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Abstract

The cyprote.rone acetate (CPA) is an antiandrogen drug that is used in the treatment of prostate cancer, which is related to drug-induced liver injury (DILI). The aim of this study the effect of the water extract of one of the local dates on the side effects of using cyproterone acetate on liver tissue in white mice. Forty from albino mice male were divided into 8 equal groups received orally in one ml as follows. Group 1: distilled water passive control, group 2: corn oil positive control, group 3: received with 5 mg/kg body weight CPA , group 4: received, with 20 mg/kg CPA, group 5: received with 5 mg/kg CPA & 60 mg/kg date palm extract, group 6: received, with 5 mg/kg CPA & 120 mg/kg date palm extract, group 7: received , with 20 mg/kg CPA & 60 mg/kg date palm extract, group 8: received with 20 mg/kg CPA & 120 mg/kg date palm extract lasted for 21 days.

Showed a histological study of the liver remarkable degeneration of hepatocytes associated with interstitial necrosis and blood vessel congestion.

The current study proved that the water extract of dates has a weak effect in the repair of damage in the liver tissue to treatment for the low dose of cyproterone acetate only.

Keywords: *Cyproterone acetate, Date palm fruit, hepatotoxicity, histopathology.*

Introduction

CPA is synthetic progesterone and antiandrogenic component administered It is used to treat many physical conditions in pro.state cancer and also in breast cancer, serious acne, womanly hirsutism, precocious puberty, hypersex.uality [(1).]

Drugs can have direct toxic effects (dose-dependent) or elicit hypersensitivity or metabolic distinctive reactions (dose-independent) that can take place at any time during the course of therapy [(2).] Hepatotoxicity signs resulting from the use of both steroidal androgens and nonsteroidal antiandrogens causes many cases asimmunoallergic cytotoxic reactions, cholestasis, auto.immune hepatitis [(3 &4)], acute hepatitis, fulminant hepatic fail [(5)] cirrhosis[(1)]and ultimately, CPA has been imputed a hepatocellular mutagenic capacity leading to hepato-carcinogenesis[(7)]. Many treatments are for natural products can use because they are cheap and easy to obtain. The Prophet Mohammed (Peace Be upon Him) recommended the use of natural products as medicine for certain diseases[(8)]. In traditional medicine, herbal medicines are widely used around the world, where palm pollen grains are widely used as an anti-hepatotoxicity[(9&10)].

Pho.enix dac.tylifera L. plant is one of the elderly cultivated plants in the Middle East and North Africa. The fruits of dried dates contain 8 phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, and ferulic acid [(11)&(12).] In addition, date fruits contain

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Ascorbic acid, β-carotene, nicotinic acid, riboflavin and thiamine [13]. and include twenty-one free amino acids (leu.cine, α-alanine, and proline were predominant), and the amides aspargine and glutamine were particular in P. dactylifera [14]. Another study indicated that date palm fruit contains cholesterol, campesterol, stigmasterol, β-sitosterol, and fucosterol [15]. Every date types are a user provenance of natural antioxidants It can be considered effective food [16] since date fruit extract had a powerful antioxidant and antimutagenic specialty [17].

Vari.ous studies have that the date fruit extract has been shown to ameliorate liver damage in rat, inhibit swelling and tumors, suppress the growth of Streptococcus pyogenes and improve sperm parameters [18) &(19).

Materials and Method

Preparation of extract

Fresh date palm fruits (*Pho.enix dactylifera L., Palmae*) were provided from a local market in Hilla (Babil, Iraq), dried at room temperature, and were manually isolated from the pits. The flesh of the dried *P. dactylifera* fruits was ground. About 650 g of the powder was soaked in 2 L of cold distilled water. After 24 h, the solution was filtered and evaporated under vacuum and dried to a constant weight using a freeze-drier. The dry extract of the fruit was dissolved in distilled water instantaneously before treated the mice [20].

Preparation of cyproterone acetate:

Obtained cyproterone acetate anti-androgen from local pharmacies and called Androcur as a trading name and equipped from a company of a subsidiary of Filiale de Schering AG Germany, 20 mg concentration for each disc. Dissolved the drug used in this study in absolute ethyl alcohol and left exposed to the air until drought then added to the powder pure corn oil. solute each disc in 12.5 ml from corn oil to obtained 20 mg/ml this study in absolute ethyl alcohol and left exposed to the air until drought then added corn oil and calculated required concentrations to conduct experiments depending on the dose given to human[21].

Animals of the experiment:

In the current study, 40 male white mice have used range ages from used 2-3 months. The mice were provided with eating and water *adlibitum.*

Experimental protocols:

Mice were randomly divided into eight groups each contains 5 animals treated daily with one milliliter orally as follows:

1-First control group: treated with distilled water for 21 days, as a negative control.

2-Second control group: treated with corn oil for 21 days, as a positive control.

3-Third group: treated with cyproterone acetate 5 mg/kg/BW for 21 days.

4-Fourth group: treated with cyproterone acetate 20 mg/kg/BW for 21 days.

5-Fifth group: treated with cyproterone acetate 5 mg/kg/BW and crude date extract 60 mg/kg/BW for 21 days.

6-Sixth group: treated with cyproterone acetate 5 mg/kg/BW and crude date extract 120 mg/kg/BW for 21 days.

7-Seventh group: treated with cyproterone acetate 20 mg/kg/BW and crude date extract 60 mg/kg/BW for 21 days.

8-Eighth group: treated with cyproterone acetate 20 mg/kg/BW and crude date extract 120 mg/kg/BW for 21 days.

Animals were sacrificed 24 hours after of the last dose, use diethyl ether to drugged mice, open the abdominal cavity and remove the liver, then fixed the fresh small pieces of each mouse liver in the formalin solution 10 % until the histological preparation.

Histological study:

Ordinary histological processing is prepared for the liver in order to study the histopathological changes that may be found in the experimental groups as compared with negative and positive control groups. According to[22], the processing steps and staining technique was as follow: small pieces of livers were dehydrated using a graded ethanol series, subsequently emb.edded in paraffin, wax blocks were cut by the microtome to prepare 5μm thick sections and stained with hematoxylin after deparaffinization of sections in xylene and hydrated in progressive descending ethanol series and stained with eosin after the washing and differentiate,

then wash again, dehydrate, cleared in xylene and mount with Canada balsam on glass slide for light microscopic examination.

Results and Discussion

Several studies have indicated in Liver enzyme aberration have in experimental animals treated with cyproterone acetate [23]. This our study showed treated mice of 5 mg/kg body weight of CPA (group 3, fig. 1) revealed degeneration and necrotic changes in hepatocytes, sections of liver of CPA 20 mg/kg/BW treated mice (group 4, fig. 2) showed congested blood vessels, necrosis in hepatocytes and moderate inflammatory cell infiltration in the portal triad as compared to other treated groups. The hepatotoxicity depend on both the dose and the duration of xenobiotics exposure will impact the type and grade of toxicity, there is often susceptibility to the toxicity based on the intralobular site of hepatocytes for xenobiotics that immediately affect the liver and hepatocellular necrosis, evidence of necrosis is generally apparent within forty-eight hour or previously[24].

One study noted an increase in serum aspartate aminotransferase (AST) and alanine aminotransferase. (ALT) activities in all patients with inprogress prostate cancer who are treated with CPA-induced liver damage, in 91% of those cases, the type of hepatic injury was hepatocellular damage. This damage is frequently involved hepatocytes damage that is associated with an elevated ALT level (25). It is generally synched progress that hepatocellular hypertrophy may be a serious qualitative metric, but classify the severity of hypertrophy is less accurate than relying on liver weight or quantitative measuration of enzyme induction. There is a substantial relationship between hepatotoxicity and enzyme induction with clinical pathology measurements are described (26). Changes may occur in liver histology without enzyme reduction but include fluid aggregation, fatty change in hepatocytes, inflammatory cell infiltration, fibrosis, and probably granuloma formation (27).

Examination of stained liver sections of animals treated with 5 mg/kg/BW of CPA and crude aqueous palm date fruit extract 60 mg/kg/BW (group 5, fig.3) showed a noticeable degeneration in hepatocytes, sections of the liver of CPA 5 mg/kg/BW and crude palm date fruit extract 120 mg/kg/BW treated mice (group 6, fig. 4) revealed congested blood vessels and necrosis of hepatocyte as compared with CPA treated mice with reducing severity in two groups. “The mechanism by which the aqueous date palm fruit extract induces its hepatoprotective activity versus oxidative damage caused by any drug is not clear. However, it is potential that polyphenolic compounds (flavonoids, anthocyanins, and phenolic acids), and trace elements (selenium, copper, zinc, and manganese), an extension to vitamin C present in the date palm fruit are the responsible compounds for this protection (28; 29; 30).

One of the studies conducted on the aqueous extract of date fruits It acts as an antioxidant and the antimutagenic activity, Where this extract on the inhibition of lipid peroxidation and protein oxidation and also by the aptitude to scavenge superoxide and hydroxyl radicals [31]. In addition to that, there are many studies indicate the hepatoprotective activity of any drug is the ability of its components to block the aromatase activity of cytochrome P-450. On that basis, it is proposed that flavonoids in Phoenix dactylifera could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase [32].

Tissue sections of the liver of CPA 20 mg/kg/BW and palm date fruit extract 60 mg/kg/BW treated mice (group7, fig. 5) showed congested blood vessels and severe degeneration in hepatocyte, in the liver of animals treated with CPA 20 mg/kg/BW and palm date fruit extract 120 mg/kg/BW (group 8, fig. 6) Although comparative studies indicated that dried date palm fruit with phenolic content was higher than fresh date palm fruits[33] . However, the water extract of dried palm fruit used in this study did not have the ability to preserve the liver from the toxic effect of the drug CPA especially at a high dose. The direct effect of cyproterone is attributed to increased of hepatocytes of placental glutathione S-transferase, which are believed preneoplastic elements[34-35]. Where it worksGrowth Factor-beta 1 (TGF) expression on the induction of apoptosis might account for both the liver damage and the expansion of liver tumors observed after giving a drug of CPA [36].

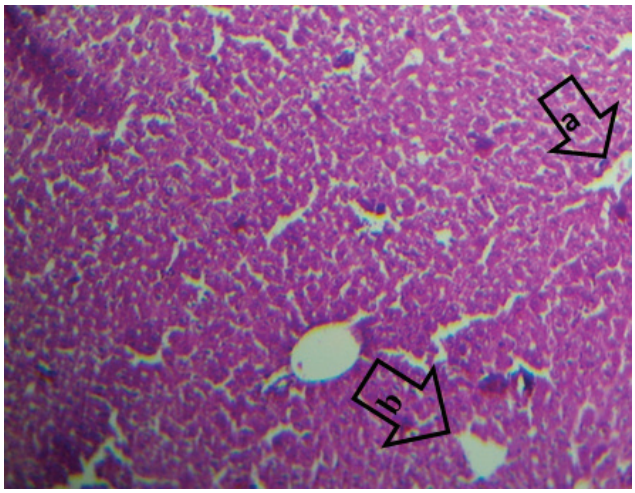

Figure-1: Histological liver section of (CPA) 5 mg/kg, group showed (a)Marked activation kuppfere cell . (b) degeneration of the hepatocyte necrosis of hepatocyte.

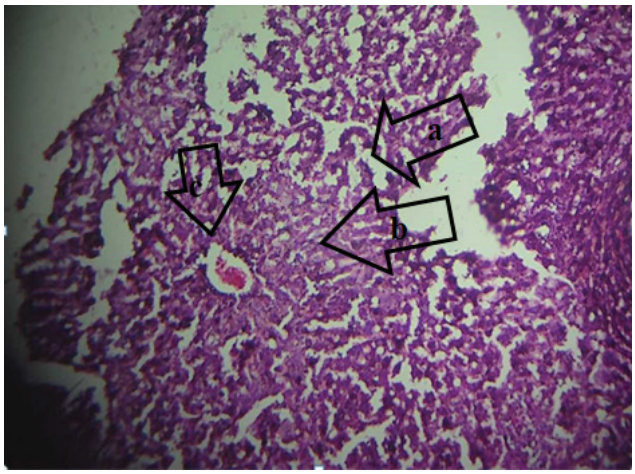

Figure-2: Histological liver section of (CPA) 20 mg/kg group showed (a) sever degeneration of hepatocyte (b).hypertrophied hepatocytes with deeply stained shrunken nuclei .(c) congested blood vessels.

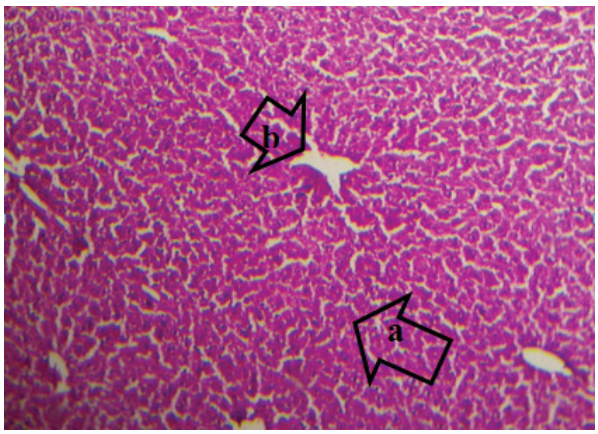

Figure-3: Histological liver section of (CPA) 5 mg/kg + 60 mg/kg date palm extract, group showed (a) Some hepatocytes were free from nuclei and others contained pyknotic nuclei.

Figure-4: Histological liver section of (CPA) 5 mg/kg + 120 mg/kg date palm extract, group showed (a) moderate hypertrophy of cells and (b) moderate hemorrhagic area .

Figure-5: Histological liver section of (CPA) 20 mg/kg + 60 mg/kg date palm extract, group showed sever degeneration of hepatocytes (b) and multihemorrhagic Areas.

Figure-6: Histological liver section of (CPA) 20 mg/kg+120 mg/kg date palm extract, group showed (a) congested blood vessel (b) necrosis of hepatocyte (c) moderate inflammatory cells infiltration in the portal triad.

Conclusion

the present study has shown that CPA has a toxic effect and some histopathological changes have been detected, so care should be taken when CPA is prescribed as antiandrogenic treatment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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