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Therapeutic effect of date palm extract on ccl4 induced HCC in rat

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the world's third leading cause of cancer death. The main risk factors for HCC are viral infection (particularly hepatitis C and B viruses) and alcohol. Herbal medicine-based therapy has become an effective therapeutic option for the treatment of many diseases, including liver cancer, in recent years. **Materials and methods:** Four groups of adult male Swiss albino rats were divided: 1st negative control group; 2nd positive group: HCC induction was done using tetra chloride carbon (CCL4) 1ml/kg was administered IP twice a week for three months to develop HCC; 3rd date palm extract: rats treated with date extract (400 mg/kg b.wt/day) after induction of HCC; 4rd cisplatin group: rats treated with cisplatin (1.5 mg/kg b.wt/ i.p). Blood, liver tissues samples were collected for some biochemical and histopathological studies. Once treatment was completed, animals were scarified with an injection of urethane (1g/kg body weight) at the end of the experiment. **Results:** Overexpression of liver enzymes was identified in Hcc cells when compared to treated groups. As these changes were accompanied by hepatic necrosis and inflammation.**Conclusions:** According to our findings, palm date extract may be useful as an anti-tumor therapeutic for HCC.

1. Introduction

The third most prevalent cancer in the globe and the fifth most frequent cancer overall is hepatocellular carcinoma. The most common causes of HCC are metabolic toxins like alcohol or aflotoxin, viral hepatitis infection (hepatitis B virus or hepatitis C virus), or both. Iron overload, fatty liver disease, and exposure to environmental toxins are other variables that contribute to the development of HCC [1].

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Recent research has demonstrated that dietary components including antioxidants play a significant impact in the development of malignancies, palm fruit alleged ability to fight cancer has also been the subject of substantial research on the anticancer and protective properties of natural compounds [2].

Traditional medicine has long recognized the many health advantages of palm fruit, which has been used to treat a variety of ailments such as bronchospasms, asthenia, menstrual cramps, insomenia, pain alleviation, and cardiovascular disease. In recent years, researchers have concentrated on the pharmacological properties of the palm fruit and its isolated components, many in vivo and in vitro studies' findings have shown that it has antioxidant and anticancer capabilities [3].

2. Material and Methods

2.1. Palm date ethanol extract preparation.

Fresh palm dates were purchased from a local market in Zagazig, Egypt. Before being used, the dates were given two washes in double-distilled water. After being oven dried, the pulp was crushed and extracted with ethanol at a 1:3 (w/v) ratio for 48 hours at 24 °C. After that, the extract was put through Millipore and Whatman filters. The final step was to condense the extracted substance and store it at -80 °C to create a thick syrup [4]

2.2. Reagents and chemicals.

Cisplatin and carbon tetrachloride (CCL4) was obtained from Sigma Chem. Co., Absolute ethanol above 99% and Sodium chloride from (Alamia, Egypt), enzyme linked immunosorbent assay kit for MMP9, caspase 3, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), catalase (CAT), superoxide dismutase(SOD), glutathione(GSH) from Sigma Chem. Co.

2.3. Experimental design.

Prior to the trial, Wistar male rats (n = 32) were acclimated for one week. Each of the four experimental groups received a random assortment of rats. The first group is the negative control group, which was left untreated. For the other three groups, carbon tetra chloride was employed at a dose of 1 ml/kg twice a week for three months to develop HCC [5]. Rats in the positive control group received no further treatment and in the palm date group received 400 mg/kg body weight per day of date fruit extract [6]. Cisplatin (1.5 mg/kg b.wt/i.p.) [7]was administered to the rats in the cisplatin group, and all groups were kept for an additional two months.

2.4. Blood and tissue sampling

After the end of the experiment each animal in each of the experimental groups were undergo anesthesia and liver tissues were dissected and blood undergo Centrifuging to obtain the serum (at 2500 rpm for 10 minutes), and it was then stored at 80 °C for later analysis [8]. liver tissues was preserved for 24 hours in 10 percent neutral buffered formalin for histological studies([9].

2.5. Biochemical studies

Serum samples undergo biochemical studies using The enzyme-linked immunosorbent assay (ELISA , Sigma kit) to estimate each of liver enzymes level(ALT,AST and ALP), apoptotic (MMP9 and caspase 3) and antioxidant activity(CAT,SOD and GSH).

2.6. Histological studies

Liver tissues were dehydrated using an ethanol gradient, washed in xylene, and then fixed in paraffin wax. Tissue

blocks were deparaffinized, sectioned, stained with hematoxylin and eosin, and examined under a microscope [10].

2.7. Statistical analysis

The Statistical Package for the Social Sciences was used for all results (SPSS, version 23). The mean and standard deviation were used to express the quantitative one data (SD). The comparison was made using the one-way ANOVA test to calculate statistics between different groups and the Correlation coefficient test to determine whether a linear correlation was positive or negative by ranking different variables against each other. The level of significance was set at a P-value of <0.05 [11].

3. Results

3.1. Effect of date fruit extract and Cisplatin on liver enzymes level (ALT,AST and ALP)

Liver enzymes level were elevated in the HCC group compared with the Negative control group ($p < 0.01$) date fruit extract and Cisplatin had significantly decreased serum liver enzymes level compared with the HCC group ($p < 0.05$).

3.2. Effect of date fruit extract and Cisplatin on antioxidant activity(GSH, SOD, CAT)

GSH, SOD and CAT activities were decreased in the HCC group compared with the Negative control group ($p < 0.01$) date fruit extract and Cisplatin had significantly increased serum GSH and SOD and CAT activity compared with the HCC group ($p < 0.05$).

3.3. Effect of date fruit extract and Cisplatin on apoptotic markers (MMP9 and Caspase 3) level

There is a significant increase in serum concentration of MMP9 in HCC group as compared to negative control group. In the palm date group, palm date blocked the increase in hepatic MMP9 in rats. - Effect of palm date on active caspase 3: palm date treated group rats showed significant increase of active caspase 3 compared to HCC group and control group. This demonstrates that palm dates control many cell death pathway components, causing cancer cells to undergo apoptosis.

3.4. Effect of date fruit extract and Cisplatin on histological analysis

The histological analysis of negative control group showed normal hepatocytes, intact cell membrane, and central vein and hepatic cords formed from single cords separated by hepatic sinusoids, positive control group demonstrated liver damage, nuclei are depicted by the black arrowheads exhibiting abnormal morphology, in addition to the extensive lobular inflammatory cell infiltrations visible in the H&E-stained specimens, portal bridging of fibroblasts with lobulation and many hepatocytes revealed vacuolar degeneration and necrotic changes observed. Following the treatment with either palm date fruit extract or cisplatin, the hepatoprotective effectiveness of the polyphenols in palm date fruit extract against liver damage caused by CCL4 was assessed as section of the liver of date extract group showed hydropic and vascular degenerated of hepatocytes with normal liver pattern. Meanwhile, Cisplatin group section showed hepatic congestion, congested central vein marked hydropic degeneration and dilated sinusoids as shown in (fig.1)

Discussion

The third most prevalent cancer in the world and the fifth most common cancer overall is hepatocellular

carcinoma. The most typical problems of HCC are metabolic toxins like alcohol or aflotoxin, viral hepatitis infection (hepatitis B virus or hepatitis C virus), or both. Iron overload, fatty liver disease, and exposure to environmental toxins are other causes that contribute to the development of HCC [12]

CCl4 is a well-known promoting agent in rodent hepatocarcinogenesis [13], where phases are thought to be involved in CCl4-induced hepatotoxicity: In the first phase, CYP450 converts CCl4 to CCl3 and/or CCl3OO, which causes membrane lipid peroxidation and ultimately cell necrosis. The activation of kupffer cells, which is accompanied by the generation of proinflammatory mediators, is a component of the second stage of CCl4-induced hepatotoxicity[14]. In consistent with previous studies demonstrated the alteration of biochemical markers, hepatic antioxidant status and hepatic nucleic acid content may therefore manifest of oxidative stress and cellular DNA damage caused by CCl4, Enzymatic defence mechanisms such as catalase (CAT), superoxide dismutases (SOD), and gluthatione protect cells from free radical damage (GSH) [15]. As shown in the table(1), we revealed that palm dates increased the levels of glutathione, catalase, and superoxide dismutase when compared to the positive control group Since catalase breaks down hydrogen peroxide into water and oxygen and SOD is in responsible of removing superoxide radicals, these enzymes may help regulate the redox state of plasma.This observation perfectly agrees with those of Ceci et al [16].We focused on the potential chemotherapeutic effects of palm dates in the current study, which may have been mediated through regulating the expression of MMP9 and Caspase3 in an animal model of HCC. Palm date has been used as a herbal remedy for various aliments including cancer by the ancient Arabian, Indian and Chinese cultures . Recent studies provide proof that palm dates, which suppress cell proliferation and induce apoptosis, have a significant chemopreventive impact against liver cancer[17].

Various studies established the relevance of natural antioxidants in the amelioration of liver disease and the effect of oxidative stress in its pathogenesis. The extracellular matrix (ECM) and basement membrane are two barriers that are first broken down in the various stages of these processes, and several proteolytic enzymes are involved in this process. Matrix metalloproteinases among these enzymes are essential. MMPs are a group of enzymes that degrade basement membrane, ECM, and connective tissue macromolecules [18].This degradation results in removing physical and structural barriers which promote cell migration and invasion. In general MMP9 has been most consistently detected in a malignant tumor progression, thus down regulation the level of MMp9 is important for the prevention of malignant tumor progression [19].In our study, each of palm date treatment and Cisplatin significantly decreased the level of MMP9 comparing with HCC group thereby decreased the local spreading of tumors within the liver and suppressed tumorigenesis. In the same line to our findings, Marzieh et al. [20] investigated the inhibitory effect of palm date on MMp9 gene expression level. It was also revealed by Chen et al. [21] that Cisplatin treatment significantly decreased the level of MMP9 thereby reduced the spread of malignancies in the liver.[22]

Apoptosis, commonly known as programmed cell death, regulated activation of a preexisiting death program encoded in the genome. It is a highly coordinated form of cellular death that is crucial for the regulation of tissue cell populations during the development of tissues, hemeostasis, and regular processes like cell division and proliferation. The pathophysiology of disorders may be affected by dysregulation of apoptosis. [23].It has been demonstrated that the loss of control of normal apoptosis and the disruption of the balance between cell apoptosis and cell proliferation are the causes of cancer. [24]. The apoptosis was quantified by examination of the activity of caspase 3. Caspase 3 plays a vital role in regulating nuclear apoptosis including chromatin condensation and DNA fragmentation as well as cellular bleed [25]. Caspase 3 activation is essential in the induction of apoptosis. Caspase activation occurs via the release of cytochrome C from the mitochondria and thus, mitochondrial outer membrane permeability and cytochrome c release is directly and activates effector caspases such as caspase 3 and 7, which execute the apoptotic programme. [26].With regard to the Caspase 3, Khan et al. [17]indicated that Palm date antiproliferative activity was also associated to the induction of apoptosis, as evidenced in vitro by caspase 3 cleavage. The present study revealed a significant increase in active caspase 3 in the palm date treated group. Our results coincide with many studies, Hu et al. [27]showed the significant level of active caspase 3 as indicator of apoptosis when they used the cisplatin as natural remedy for treating HCC by decreasing cell proliferation and increasing apoptosis.

The histological results obtained in the current study, showed that CCL4 caused gross structural alterations in rat liver, with dysplastic foci have cellular atypia and tissue organization were seen scattered with hepatic parenchyma. In accordance, the study of Fujii et al. [28]showed that repeated exposure to carbon tetrachloride (CCl4) has historically been used as a model of chronic liver injury leading to cirrhosis and hepatocellular carcinoma (HCC) in rodents. Also, Elkhamesy,et al. [22]showed that administering CCL4 caused morphologic change in the liver, with substantial hepatocyte degradation, liver neoplasic cellular modification, focal infiltration of mononuclear inflammatory cells, and hepatocytes showing hepatic vacuolation.With regard to the palm date, the present study showed that the majority of cells which exhibited neoplastic suffered from apoptosis in the group of rats were treated with palm date. This also can be explained by Gad et al. [29]whom reported that the groups of rats were treated with palm date have a significant reduction in the number and size of the nodules induced by CCL4 an large number of regular hepatocytes were observed. Also Fatani et al. [30] showed that rats with HCC and treated with palm date revealed better reservation of the normal liver architecture and rare generalized vacuolization of the cytoplasm of hepatocytes, with apparently normal nuclei very few inflammatory cells infiltration.

Conclusions: In summary, the data provided here demonstrate that palm dates significantly reduced the number and incidence of hepatic nodules in CCL4-treated rats' livers. This

inhibition was associated to induced apoptosis, decreased cell proliferation, decreased oxidative stress, and decreased expression of inflammatory markers.

Participant consent

not required.

Not applicable Consent for Publication

Contribution of the author

All authors had input to the study's conception and design. The material preparation, data collection, and analysis were handled by Nabila Zein, Amira Eladly**.** The manuscript's first draught was written by Nabila Zein and all of the writers gave input on earlier draughts. All authors read and gave their approval to the final draught.

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Contrary Interests

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Table 1: Biomarkers parameters

	ALT	***AST***	***ALP***	***MMP9***	***Caspase 3***	***CAT (U/gp)***	***SOD (U/gp)***	***GSH (U/gp)***
negative control group	50.79±4.38^{**a}	66.68±9.82^{**a}	99±2.8^{**a}	22.4±1.04^{**a}	29.26±1.18^{**a}	.2±0.6^{**a}	6.6±0.64^{**a}	0.7±1.04^{**a}
positive control group	244.76±14.57	269.78±13.60	318.2±31.9	119.6±4.1	40.26±1.18	2.5±0.1	4.5±0.18	0.4±4.1
palm date group	96.76±4.95^{**b}	155.25±14.18^{**b}	197.2±20.3^{**b}	62.0±3.7^{**b}	76.53±3.19^{**b}	3.3±0.18^{**b}	5.5±0.15^{**b}	0.6±2.2^{**b}
Cisplatin group	94.6±10.8^{**b}	99.5±12.7^{**b}	147.2±15.4^{**b}	42.0±2.3^{**b}	88.53±1.14^{**b}	3.5±0.16^{**b}	5.8±0.19^{**b}	0.6±3.7^{**b}

Table 1 shows the results of an analysis of serum samples from rats treated with palm date extract and cisplatin after CCl4-induced HCC. Levels of liver. enzymes (ALT), AST, and ALP, antioxidant activity (GSH), SOD, and CAT, apoptotic markers (MMP9), and caspase 3 The values were expressed as mean standard deviation. The statistical significance of the comparisons between the four groups was represented by a P-value of ≤0.05 indicating a significant difference and a P-value of ≤0.001indicating a highly significant difference. The letters (**a) indicate a highly significant difference between the negative and positive groups. (**b) indicates a highly significant difference between treatment and control groups.

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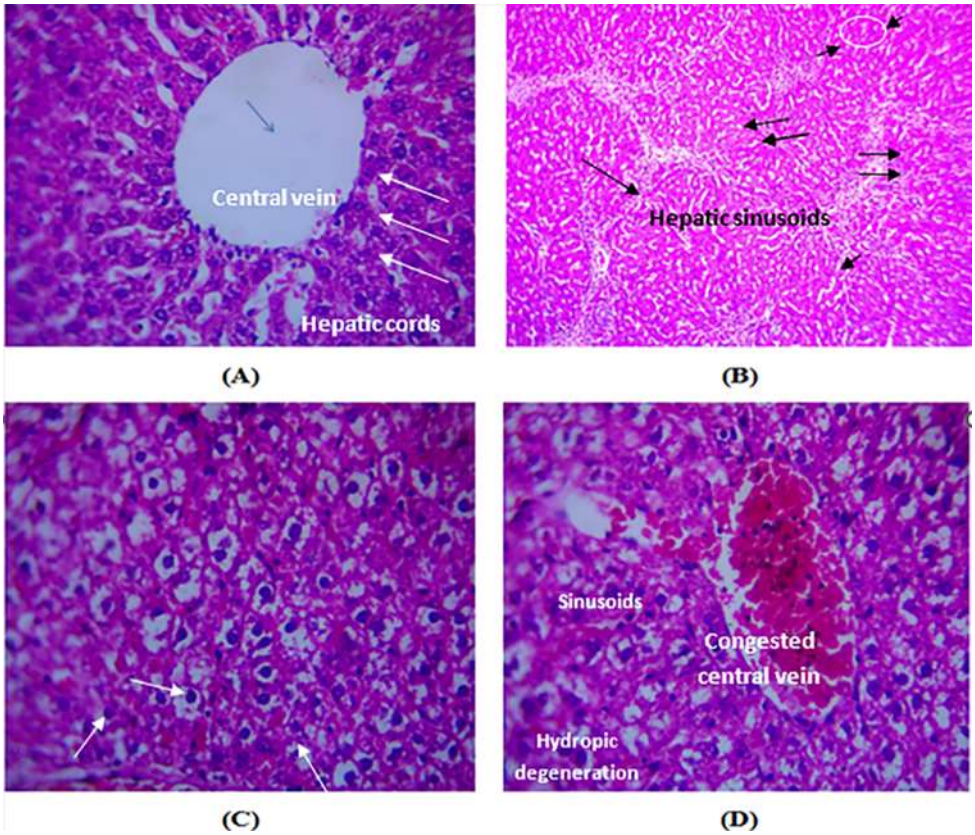

Fig(1): Histopathological changes in liver tissues; negative control group (photomicrographs A), Positive control group (photomicrographs B), palm date group (photomicrographs C) and Cisplatin group (photomicrographs D). Slides were examined using H&E stain with magnification X400.