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Original Research Article

Evaluation of Hepatoprotective Effect of Heart of Palm (Palmito) Extract against CCl_4 Induced Hepatotoxicity in Adult Male Rats

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Abstract

The aim of the present investigation was to examine the effects of heart of palm (palmito) aqueous extract in hepatotoxicity induced by carbon tetrachloride (CCl_4) in Albino adult male rats. Thirty rats were used and divided randomly into five groups, each of which contain six animals, group I was served as a negative control, received no treatment, the group II was treated with oral CCl_4 at a dose of 0.5 ml/kg body weight to induce hepatotoxicity every other day for seven day, group III, IV and V received CCl_4 in similar manner with aqueous extract of palmito daily intake at different doses (100,250,500 mg/kg) respectively for two weeks. Blood samples obtained from orbital sinus puncture by use special capillary tube at pre-treatment and at the end experiment for biochemical analyses : activities of liver functions enzymes were assessed by estimating alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total, direct and indirect bilirubin (TSB, DB and IDB respectively), total protein (TSP), albumin and total lipid. The result indicated that palmito has the ability to protect hepatic tissue against the toxicity induced by CCl_4 revealed through significant reduction of serum activities of liver enzymes and in the concentrations of TSB, DB and total lipid, and with a significant increase of serum concentrations of total protein and albumin. In conclusion, palmito can be proposed to protect the liver tissue against CCl_4-induced liver damage in rats.

Keywords

Antioxidant

CCl_4

Hepatoprotective

Liver

Palmito

Introduction

The status of herbal medicine has been fast gaining ground all over the world during the last few decades, mainly due to the general belief that herbal drugs are without any side effects besides being cheap and locally available (Nitha et al., 2011; Kumar et al., 2012). The palmito, known as "heart of palm", is the central part or heart of some wild varieties of palm trees, it's one of such plant grown in many localities in

arid and semi-arid regions of the world (Abohatem et al., 2011) is considered as a good source of energy, it contain vitamins, a group of elements like phosphorus, iron, potassium, and significant amount of calcium (El- Gazzar et al., 2009) . The information accrued in the past four decades suggest that dates possess diverse medicinal uses including antihyperlipidemic, anticancer, gastroprotective, nephroprotective, anti-inflammatory, and hepatoprotective effects (Baliga et al., 2011). It is also used in the treatment for sore throat, cystitis gonorrhea, and edema and to counteract alcohol intoxication (Al-Daihan and Bhat, 2012). Besides fruit, the date palm over the centuries has also provided a large number of other products which have been extensively used by man in all aspects of daily life (Agoudjil et al., 2011). *Euterpe edulis* (known as Palmetto) is the most important widely used species which locally (Shimizu et al., 2011).

Liver disease is an acute or chronic damage to the liver, usually caused by infection, injury, exposure to drugs or toxic compounds, an autoimmune process, or by genetic defect. Chemical such as carbon tetrachloride (CCl_4) catabolism radicals induced lipid peroxidation, damage the membranes of liver cells and organelles, and causes the swelling and necrosis of hepatocytes and result to the release of cytosolic enzymes such as AST, ALT and ALP into the circulating blood. These enzymes are used as the indicators of chemically induced liver damage (Drotman and Lawhan, 1978). The CCl_4 is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effect of CCl_4 is largely due to its active metabolite, trichloromethyl radical accumulation in the liver (Thangakrishnakumari et al., 2012).

The present study was designed to elevate the possible hepatoprotective effect of different dose of *Euterpe edulis* against CCl_4 induced liver damage in experimental rats.

Materials and methods

Collection and authentication of plant

For the present work the fresh plant materials of *Euterpe edulis* were collected from Ba'aquba city/Dyala /Iraq. The plant was positively identified by Biologist literature in Biological department /College of Science /University of Mosul / Iraq. The plant was washed with tap water and dried in shade at room temperature, and then it cut into small parts.

Preparation of *Euterpe edulis* extract

A bout (300gm) of dried plant were added to (900 ml) of distilled water (1/3, w/v) then crushed by a blender at room temperature for overnight using shaker. After that, the mixture was filtrated through glass woods. The extract was subjected to freezing at (- 20 C°) and thaw cycle for a period of three days (Kreader et al., 2004), then ultrasonic system was used to breakage cells walls (Robyt and White, 1987). The mixture were collected and concentrated to suspended solution under reduced pressure using Lypholyzer system.

Experimental design

Healthy male albino rats weighting between (220-350g) were obtained from the animal house in the medicine faculty / Mosul University / Iraq. Rats were housed under standard laboratory condition, light and dark cycles of 12h, in a polypropylene cages and allowed free access to feed and tap water under strictly controlled pathogen free conditions with room temperature (25±2ºC), humidity (50±5%).The animals were randomly divided into five groups, comprising of six rats in each.

Group I: Received (0.5ml/kg) body weight of distilled water orally for 2 weeks and used as the normal control. The animals in all groups were killed by anesthetic ether on the day 15.

Group II: Received (0.5ml of mixture of 1:1 V/V in a corn oil/kg) body weight CCl_4 orally by gavages tube to all groups except normal control group to induce hepatotoxicity on 1^{st}, 3^{ed}, 5^{th} and 7^{th} days of experiment (Kapur et al., 1994).

Group III, VI and V received CCl_4 in similar as group II and they were treated daily with different doses of crude cold aqueous extract of *Euterpe edulis.*

Group III: Received (0.5ml/kg) body weight crud cold aqueous extract of *Euterpe edulis* daily oral intake at the dose of (100mg/kg) for 14 days.

Group IV: Received (0.5ml/kg) body weight crud cold aqueous extract of *Euterpe edulis* daily oral intake at the dose of (250mg/kg) for14 days.

Group V: Received (0.5ml/kg) body weight crud cold aqueous extract of *Euterpe edulis* daily oral intake at the dose of (500mg/kg) for 14 days.

Blood samples from all groups were collected weekly from Orbital sinus puncture by use special capillary tubes, left stand in serum tubes for (30 minutes) to be coagulated. Serum samples were collected by centrifugation at (3000 rpm) for (20 minutes) at room temperature. The clear, non-haemolysed sera was separated and stored at (- 20 C°) for measurements of biochemical analyses of liver functions (Atta et al., 1983).

Biochemical analyses

The AST and ALT activities were measured spectrophotometrically by using the method of Reitman and Frankle (Anderson and Cockayne, 1993). ALP was measured by the method devised by Bowers and McComb, which allows calculation of ALP activity basing on the molar absorptive of P-nitrophenol (Tietz, 1999). Total serum protein (Bishop et al., 2005) and serum albumin was determined quantitively by colorimetric method used bromocresol green (Doumas et al., 1971).The total proteins minus the albumin gives the globulin. The bilirubin and conjugated bilirubin were determined as described by (Bishop et al., 2005). The unconjucated bilirubin concentrations were calculated as the difference between total and conjucated bilirubin concentrations. The total lipid determined by heated the serum with concentrated sulphuric acid, then react the mixture with phosphovaniline reagent (Toro and Ackermann, 1975).

Statistical analysis

The data presented in this investigation were expressed as the mean ± Standard error (mean ± SEM) of the mean. The significant difference among the means has been statistically analyzed by one-way analysis of variance ANOVA, $p<0.05$ was considered as statistical significance using SPSS Software (Snedecor and Cochran, 1986).

Results and discussion

Effect of palmito aqueous extract on body weight of control and in CCl_4 induced hepatic damage in rats

No animals died during the study and no clinical and behavioral changes were observed in any of the animals after administration of both CCl_4 intoxicated and the palmito extract. Fifteen days after treatment with palmito extract, there were significant increased in the weights of bodies in the four treated-groups of rats compared to those in the control group especially in group II in the first and second weeks as shown in Table 1.

Table 1. Effect of palmito aqueous extract on body weight of control and in CCl_4 induced hepatic damage in rats.

Treatment	Body weight (gm) (mean ± SEM)	
	1 st week	2 nd week
Group I (n=6)	183.2±21.11 a	239.4±26.40 a
Group II (n=6)	320.9±36.97 b	327.8±41.81 b
Group III (n=6)	257.9±19.64 ab	255.1±20.39 ab
Group VI (n=6)	245.1±20.24 ab	262.8±21.67 ab
Group V (n=6)	265.5±24.28 b	267.1±15.85 ab

Values with different letters within a column differ significantly at ($p<0.05$), group II compared with group I and groups II, VI and V were compared with group II. **Group I** = Control group, **Group II** = CCl_4-treated group, **Group III**=Animals treated with CCl_4 intoxication prior palmito extract at dose (100 mg/kg), **Group VI** = Animals treated with CCl_4 intoxication prior palmito extract at dose (250 mg/kg), **Group V** = Animals treated with CCl_4 intoxication prior palmito extract at dose (500 mg/kg).

Effect of palmito aqueous extract on enzymes activity of serum AST, ALT and ALP in normal and CCl_4 induced hepatic damage in rats

The effects of extract at three dose levels (100, 250 and 500 mg/kg) on serum marker enzymes in CCl_4-induced hepatic injury are shown in Table 2. Hepatic injury induced by CCl_4 caused significant rise ($p<0.05$) in marker enzymes AST, ALT and ALP in CCl_4 intoxicated group (group II) in comparison with control group (group I). Administration of palmito extract at three different dose levels attenuated the

increased levels of the serum enzymes, produced by CCl_4, for both two weeks and caused a subsequent recovery towards normalization especially at the second week.

Carbon tetrachloride (CCl_4) is commonly used to induce liver damage that histological resembles viral hepatitis. Toxicity begins with the change in endoplasmic located in the intracellular structures. The toxic metabolic CCl_3 radical ($\cdot CCl_3$) is produced and further reacts with oxygen to give trichloromethyl peroxy radical. Cytochrome P450 is the enzyme responsible for this conversion. The most serious consequence of free radical-induced liver injury is lipid peroxidation, and it has been found that, free radical can cause oxidative damage to cellular proteins and alter cellular function (Johnston and Kroening, 1998; Thangakrishnakumari et al., 2012). This leads to the pathological changes such as depression of protein synthesis, elevation levels of serum marker enzymes. The biochemical evidences presented in this study clearly demonstrated the state of oxidative stress-induced hepatic tissue damage by CCl_4 treatment, manifested by the leakages of ALT, AST, and ALP in serum (Okuno, 1986). Therefore, the results of group II had a significant elevation in the activities of ALT, AST and ALP compared to the group I, this may return to the treatment with CCl_4, which is considered as a potent hepatotoxic agent, and these enzymes are highly sensitive to liver injury and they released in significant quantities in the serum after any oxidative stress in the liver (Uskokovic- Marcovic et al., 2007). Serum ALP also increased significantly for the hepatocellular injury and cholestasis so large quantities of this enzyme were released. These results are in agreement with (Pari and Karthikesan, 2007; Abd and Al-Baghdadi, 2009) which indicated the increase permeability, damage, and necrosis of hepatocytes.

In another side the results of the groups III, VI and V revealed that the values of serum ALT, AST and ALP decreased significantly compared with group II. Therefore, the reduction in activities of the enzymes by palmito extract is an indication of stabilization of serum membrane as well as repair of hepatic tissue damage caused by CCl_4. Serum levels of transaminases which are sensitive indicators of liver-cell injury and are helpful in recognizing hepatocellular diseases. These enzymes return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Frank et al., 2012). The presence of functional antioxidant compounds (such as selenium, polyphenols, phytohormones and carotenoids) in the date palm fruit in the "Rutab" and "Tamr" stages might be responsible for lowering the values of hepatic enzymes activity (Al-Sayyed et al., 2013). In addition (Saafi et al., 2010) found a significant increase in the hepatic activity of these enzymes after treatment of adult male rats with an aqueous extract from date palm fruit (4 ml/kg body weight) for 2 months. One study found that feeding a mixture of date palm fruit to adult male rats treated with lead acetate (to induce lipid peroxidation) for 7 weeks enhanced the activity of serum glutathione-S-transferase activity (Al-Sayyed et al., 2013).

Effect of palmito aqueous extract on serum total protein (TSP), albumin and globulin in normal and CCl_4 induced hepatic damage in rats

The total protein, albumin and globulin concentrations were significantly decreased at (p<0.05) in CCl_4 intoxicated rats (group II) when compared with control (group I). The effects of extract at the different doses on CCl_4-induced hepatic injury are shown in Table 3, which noted that the (100, 250 and 500 mg/kg) body weight of palmito extract (groups III, VI and V) gives a significant decrease when compared with control group while gives a significant increase in comparison with group II for first and second week (Table 3).

Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum proteins, hypoproteinemia is a feature of liver damage due to significant fall in chronic liver disease (Hussein et al., 2007). The reduction in the level of TSP, albumin and globulin in the group II indicated the effect of CCl_4 on liver functions. The hypoproteinemic which was observed after CCl_4 ingestion but the trend turns towards normal after palmito extract treatment which was significant elevation in the level of TSP, albumin and globulin in different doses of palmito extract groups for both first and second week, this may be due to treatment with the extract which preserve the structural integrity of the liver from the toxic effects, so the significant changes in these parameters indicated antihepatotoxic effect and the ability of palmito extract to stimulate the regeneration of hepatic tissue which increase protein synthesis in damaged liver and improve the functional status of the liver cells (Thangakrishnakumari et al., 2012).

Table 2. Effect of palmito aqueous extract on enzymes activity of serum AST, ALT and ALP in normal and CCl_4 induced hepatic damage in rats.

Treatment	ALT(U/L) (mean ± SEM)		AST (U/L) (mean ± SEM)		ALP (U/L) (mean ± SEM)	
	1st week	2nd week	1st week	2nd week	1st week	2nd week
Group I (n=6)	346.2±27.84 a	265±5.68 a	401.5±29.43 b	148±2.91 a	544.5±21.8 b	555.7±11.28 a
Group II (n=6)	493.1±27.45 c	444.7±16.44 c	490.7±19.67 c	344.8±8.39 c	614.3±7.40 c	1284±13.31 d
Group III (n=6)	403±11.44 ab	319.1±26.97 b	296.3±34.93 a	155.4±13.49 a	410.4±30.66 a	1065.7±8.56 c
Group IV (n=6)	388.8±16.03 ab	305.8±12.54 b	375.7±24.41 b	243.7±48.56 b	490.6±26.53 b	702.3±6.39 b
Group V (n=6)	414.1±11.82 b	230.5±9.05 a	433±6.97 bc	166.4±4.48 a	525.3±9.99 b	503.1±7.08 a

Values with different letters within a column differ significantly at (p<0.05)**,** group II compared with group I and groups II, VI and V were compared with group II**. Group I** = Control group, **Group II** = CCl_4-treated group, **Group III** = Animals treated with CCl_4 intoxication prior palmito extract at dose (100 mg/kg), **Group VI** = Animals treated with CCl_4 intoxication prior palmito extract at dose (250 mg/kg), **Group V** = Animals treated with CCl_4 intoxication prior palmito extract at dose (500 mg/kg).

Table -3 Effect of palmito aqueous extract of on serum total protein (STP), albumin and globulins in normal and CCl_4 induced hepatic damage in rat.

Treatment	STP (g/dL) (mean ± SEM)		Albumin (g/dL) (mean ± SEM)		Globulin(g/dL) (mean ± SEM)	
	1st week	2nd week	1st week	2nd week	1st week	2nd week
Group I (n=6)	9.02±0.17 d	9.04±0.71 c	2.80±0.01 c	2.11±0.12 b	6.89±0.31 d	6.80±0.19 a
Group II (n=6)	4.78±0.22 a	4.11±0.41 a	2.12±0.24 a	1.53±0.24 a	2.06±0.16 a	1.74±0.39 a
Group III (n=6)	6.51±0.43 c	5.99±0.08 bc	2.52±0.18 b	2.97±0.005 cd	3.12±0.17 b	2.09±0.31 ab
Group IV (n=6)	5.54±0.01 b	5.83±0.21 b	2.34±0.01 ab	3.37±0.23 d	3.20±0.01 b	2.65±0.21 b
Group V (n=6)	5.19±0.07 ab	5.43±0.15 b	2.60±0.15 b	2.73±0.09 c	4.23±0.46 c	2.70±0.14 b

Values with different letters within a column differ significantly at (p<0.05)**,** group II compared with group I and groups II, VI and V were compared with group II**. Group I** = Control group, **Group II** = CCl_4-treated group, **Group III** = Animals treated with CCl_4 intoxication prior palmito extract at dose (100 mg/kg), **Group VI** = Animals treated with CCl_4 intoxication prior palmito extract at dose (250 mg/kg), **Group V** = Animals treated with CCl_4 intoxication prior palmito extract at dose (500 mg/kg).

Effect of palmito aqueous extract on serum total bilirubin (TSB), direct bilirubin (DB), and in direct bilirubin (IDB) in normal and CCl_4 induced hepatic damage in rats

The results revealed that group II had a significant elevation (p<0.05) in the levels of serum TSB, DB and IDB compared to the group I and each of the groups III, VI and V. In another side the results in these groups which have a different doses of palmito extract (groups III, VI and V) revealed that the values of TSB, DB and IDB decreased significantly in comparison with the group II for both first and second week, as shown in Table 4. Serum bilirubin is considered as one of the true test of liver functions since it reflects the ability of the liver to take up and process bilirubin into bile. Elevated levels may indicate several illnesses. Hyperbilirubinemia may result from the production of more bilirubin than the liver can process, damage

to the liver impairing its ability to excrete normal amount of bilirubin or obstruction of excretory ducts of the liver (Olaleye et al., 2010). High significant levels of TSB in CCl_4 treated rats may be due to rapidly produce lipid peroxidation of hepatocytes membranes by the action of free radicals (Lee et al., 2004). This may have resulted in hyperbilirubinemia. The significant reduction in the level of total bilirubin in the serum of palmito extract treated rats suggested the hepatoprotective potential of *Euterpe edulis* extract against CCl_4 intoxication.

Effect of palmito aqueous extract on serum total lipid in normal and CCl_4 induced hepatic damage in rats

The serum total lipid concentration of the experimental groups which received a (100, 250 and 500 mg/kg) body weight of palmito extract were significantly increased compared with the control group (p<0.05), while the value of serum total lipid in these groups decrease significantly in comparison with the CCl_4 induced hepatic damage group which are shown in Table 5.

Table 4. Effect of palmito aqueous extract on serum total bilirubin (STB), direct bilirubin (DB) and indirect bilirubin (IDB) in normal and CCL_4 induced hepatic damage in rats.

Treatment	STB (µ mol/L) (mean ± SEM)		DB (µ mol/L) (mean ± SEM)		IDB (µ mol/L) (mean ± SEM)	
	1st week	2nd week	1st week	2nd week	1st week	2nd week
Group I (n=6)	6.34±1.05 a	7.55±0.83 b	5.43±1.04 a	6.04±1.01 b	5.09±0.56 b	4.13±0.84 a
Group II (n=6)	68.85±5.75 c	11.98±2.21 c	16.31±2.09 d	8.44±1.35 c	13.54±0.91 d	6.98±0.85 b
Group III (n=6)	12.14±0.22 a	9.78±0.35 bc	9.28±0.36 bc	2.44±0.20 a	2.85±0.28 a	4.13±0.78 ab
Group VI (n=6)	18.76±1.01 b	3.93±0.51 a	8.59±0.42 b	1.81±0.333 a	8.53±0.17 c	2.11±0.81 a
Group V (n=6)	20.31±0.86 b	3.01±0.32 a	11.35±0.49 c	1.44±0.19 a	5.57±0.50 b	4.11±0.91 A

Values with different letters within a column differ significantly at (p<0.05)**,** group II compared with group I and groups II, VI and V were compared with group II**. Group I** = Control group, **Group II** = CCl_4-treated group, **Group III** = Animals treated with CCl_4 intoxication prior palmito extract at dose (100 mg/kg), **Group VI** = Animals treated with CCl_4 intoxication prior palmito extract at dose (250 mg/kg), **Group V** = Animals treated with CCl_4 intoxication prior palmito extract at dose (500 mg/kg).

Table 5. Effect of palmito aqueous extract on serum total lipid in normal and in CCl_4 induced hepatic damage in rats.

Treatment	Total lipid (mg/dL) (mean ± SEM)	
	1 st week	2 nd week
Group I (n=6)	268.2±13.15 a	560.2±7.24 a
Group II (n=6)	504.8±19.86 d	645.9±13.37 b
Group III (n=6)	315.7±10.35 b	580.0±37.5 ab
Group V I (n=6)	434.5±14.06 c	522.6±22.8 a
Group V (n=6)	323.1±7.61 b	629.2±16.14 b

Values with different letters within a column differ significantly at (p<0.05)**,** group II compared with group I and groups II, VI and V were compared with group II**. Group I** = Control group, **Group II** = CCl_4-treated group, **Group III**=Animals treated with CCl_4 intoxication prior palmito extract at dose (100 mg/kg), **Group VI** = Animals treated with CCl_4 intoxication prior palmito extract at dose (250 mg/kg), **Group V** = Animals treated with CCl_4 intoxication prior palmito extract at dose (500 mg/kg).

The total lipid concentration was significantly increased in CCl_4 group when compare with control group, while it was significantly decreased in groups III, VI and V when compare with group II after two weeks of treatments. This finding coincided with the report of (Bhathena et al., 2002; Abuelgassim, 2010). Although, (Chaira et al., 2007) reported that flesh and pit extracts of date palm fruit have free radical scavenging activities, however, the significant effect after two weeks of palmito extract on serum total lipid

level could be attributed to the antioxidant potentials of palmito extract. Another rationale explanation for the effect of palmito extract on total lipid concentration in rats could be due to prevention of oxidative stress, from this view; present findings support the report of (Saafi et al., 2010).

Conclusion

In the present study, the results demonstrate that the aqueous extract of *Euterpe edulis* plant have a potent hepatoprotective action against CCl_4 induced hepatic damage in rats.

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