Aqueous Extract of Date Fruit (*Phoenix Dactylifera*) Has Hepatocurative Effect on Carbon Tetrachloride-Induced Toxicity in Rats

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***Abstract*: Liver disease account for a reasonable percentage of medical admissions, and the synthetic drugs used for the treatment of the disease are sometimes ineffective and/or very expensive. Furthermore, the side effects associated with some of them are numerous. These and many other reasons shifted the interest of scientists for the search of plants with hepatocurative effect. Therefore, the aim of this research was to investigate the curative effect of aqueous extract of date fruit (*Phoenix dactylifera*) on rats with carbon tetrachloride-induced hepatotoxicity. The serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin (DB) and total bilirubin (TB) were measured as markers of liver disease using standard methods, and compared among group I (Negative control), group II (Positive control), group III (Fed with food + 249mg/kg of extract), group IV (Fed with food + 580mg/kg of extract), group V (hepatotoxic rats treated with 249mg/kg of extract) and group VI (hepatotoxic rats treated with 580mg/kg of extract). The results showed that administration of the various concentration of the extract to healthy rats does not cause any harm to the liver. Also, the different concentrations of the extract significantly ($P < 0.05$) reduced the level of ALT, AST, ALP, DB and TB in treated groups (group IV and V) compared to group II. These results suggest that the aqueous extract of date fruit may have hepatocurative effect against CCl_4-induced liver damage in rats. This research may open the "gate" for the use of date fruit in treating liver disease in human, especially in places such as Northern Nigeria, where date fruit is cheaply abundant.**

***Key words*: Liver, Carbon tetrachloride, Date fruit, Hepatocurative, Aqueous extract.**

I. INTRODUCTION

The liver is the largest gland of the body and second largest organ of the body [1]. It is situated in the upper right portion of the abdominal cavity [2]. This location of the liver is very essential in carrying its function [3]. Because of its multidimensional functions and strategic location, the liver is prone to many diseases. The most common are hepatitis A, B, C, D and E, liver cirrhosis and cancer. Most of these diseases are accompanied by remarkable change in biochemical parameters [1].

Liver disease has a worldwide distribution [1]. In Nigeria, liver disease accounted for 7.9% of medical admission [5]. The liver being the central focus for metabolic activities and other multifarous functions in the human body and whose damage can negatively affect almost every organ in the body, need to be protected from being damaged or need to be properly cured when infected. Despite the efforts in developing drugs for liver disease, it is still prevalent. This is due to the fact that many of the drugs have significant side effects, and sometimes may even complicate rather than alleviate the infection. Furthermore, many of these treatments are expensive. This and many other reasons necessitate for the search of traditional herbal medicine that possess curative effects [6].

The use of plant for medicine is as old as man himself [6]. Although there is doubt in the efficiency and safety of the traditional herbal medicine, no one can argue their existence and practice in our societies. However, this doubt can be discarded if systematic research methodology is employed in evaluating the scientific basis for traditional herbal drugs (medicine). As such, many plants that are claimed to possess hepatocurative effect were tried on animals. Among such plants is Date fruit (*Phoenix dactylifera)* [7]. Date fruit, *Dabino* in Hausa language, is grown in Northern Nigeria for consumption and local trade. Hence, the fruit is found in abundant quantity and cheap price. A lot of it is also imported from Niger Republic. Many researches on the medicinal effect of date fruit were conducted [8], [9], [10], [7].

Considering the abundance, availability and cheap price of this fruit in Northern Nigeria, coupled with its numerous medicinal values and the high probability of its application as hepatocurative agent, it becomes an obligation on researchers to explore it with the hope of contributing in reducing liver disease cases.

II. MATERIALS AND METHODS

A. Experimental Animals

Male and female albino rats weighing between 200g to 250g were purchased from Biological Sciences Department,

Bayero University, Kano. The rats were housed in a well ventilated animal house, and were allowed to acclimatize for one week prior to the experiment and had access to food and clean water *ad libitum*.

B. Plant Material

Date fruit- *Phoenix dactylifera* (*Dabino* in Hausa); "*Dan Agadas*" variety was bought from Babaldu junction date fruit market in Birnin Kudu Local Government Area of Jigawa State, Nigeria.

C. Preparation of Plant Material

The extract was prepared as explained by [10] with little modifications. The date fruit obtained was air dried after which the flesh was separated manually from the pit. The flesh was further dried under shed at room temperature. The dried flesh was pulverized into powder and 650g was soaked in a container of 2 litres of cold distilled water for 24 hours. The solution was filtered and the filtrate collected in a container, and evaporated at a temperature of 40°C – 50°C. The extract was stored for further use.

D. Preparation of Carbon Tetrachloride

Carbon tetrachloride was prepared as CCl_4 solution in olive oil.

E. Animal Groupings and Treatment

A total number of thirty (30) animals were used. Each group consists of five animals. The animal grouping was as follows:

Group I: Negative control (Food + water only).

Group II: Positive control (100 mg/kg CCl_4 once) administered subcutaneously as reported by [11].

Group III: Food + 249 mg/kg body weight of extract for thirty days.

Group IV: Food + 580 mg/kg body weight of extract for thirty days.

Group V: Food + 100 mg/kg of CCl_4 once only + 249 mg/kg body weight of extract for thirty days.

Group VI: Food + 100 mg/kg of CCl_4 once only + 580 mg/kg body weight of extract for thirty days.

F. Collection of Blood Samples

At the end of the experimental period (30 days), the animals were sacrificed by cervical decapitation. The blood of each animal was collected in a separate centrifuging tube. The blood samples were centrifuged and the sera were pipetted into separate well labelled containers. These sera were used in the estimation of the various biochemical parameters.

G. Determination of Biochemical Parameters

All the biochemical parameters (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, direct bilirubin and total bilirubin) were determined using Randox test kits (produced by Randox Laboratory, United Kingdom) specific for each of the parameter. The procedure was carried out according to manufacturer's protocol.

H. Statistical Analysis

Statistical Analysis was carried out using one way Anova. Minitab software was used. The values are expressed as Mean + SD. P values < 0.05 were considered as statistically significant.

III. RESULTS

Table I shows comparison between negative control group (group I) and the positive control group (group II). The result shows a significant ($P < 0.05$) increase in the level of serum AST, ALT, ALP, total bilirubin and direct bilirubin in group II compared to group I. This indicates that hepatotoxicity was induced in group II.

Table I: Comparison Of Biochemical Parameters Between Negative Control Group (Group I) And Positive Control Group (Group Ii)

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL BILIRUBIN (mg/dl)	DIRECT BILIRUBIN (mg/dl)
GROUP I	36.36^a ± 1.17	44.75^b ± 1.19	78.55^c ± 1.20	0.95^e ± 0.04	0.50^f ± 0.07
GROUP II	105.23^a ± 2.74	93.22^b ± 1.31	114.53^c ± 3.75	1.97^e ± 0.05	1.26^f ± 0.18

Values are expressed as mean ± SD
Values in the same column bearing the same superscript are significantly different at $P< 0.05$, n=5

Table II shows the results of biochemical parameters (AST, ALT, ALP, direct bilirubin and total bilirubin) in the negative control group (group I), positive control group (group II), group that were administered 249 mg/kg of the extract (group III) and the group that were administered 580 mg/kg of the extract (group IV). The result shows no significant ($P > 0.05$) difference in the level of biochemical parameters between group III and I. There is also no significant ($P > 0.05$) difference in the level of biochemical parameters between group IV and I. All these indicate that both 249 mg/kg and 580 mg/kg of the extract may have no harmful effect on the liver.

Table II: Comparison Of Biochemical Parameters Between Negative Control Group (Group I), Positive Control Group (Group Ii), Group Administered 249 Mg/Kg Of Extract (Group Iii) And Group Administered 580 Mg/Kg Of Extract (Group Iv)

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL BILIRUBIN (mg/dl)	DIRECT BILIRUBIN (mg/dl)
GROUP I	36.36^{a} ±1.17	44.75^{a} ±1.19	78.55^{a} ± 1.20	0.95^{a} ±0.04	0.50^{a} ±0.07
GROUP II	105.23^{abc} ± 2.74	93.22^{abc} ±1.31	114.53^{ab} ±3.75	1.97^{ab} ±0.05	1.26^{abc} ±0.18
GROUP III	35.28^{b} ± 1.71	47.54^{b} ± 1.32	80.04^{b} ± 2.06	0.98^{b} ± 0.09	0.49^{b} ±0.03
GROUP IV	37.73^{c} ±1.75	45.58^{c} ± 1.15	72.65^{ab} ± 1.16	0.97^{c} ±0.10	0.49^{c} ±0.15

Values are expressed as mean ± SD
Values in the same column bearing the same superscript are significantly different at $P < 0.05$, n=5

Table III shows the effect of oral administration of aqueous extract of *Phoenix dactylifera* on biochemical parameters in hepatotoxic group treated with 249 mg/kg of extract (group V) and hepatotoxic group treated with 580mg/kg of the extract (group VI).The result shows significant ($P < 0.05$) decrease in the level of AST, ALP, ALT, Direct bilirubin and Total bilirubin in hepatotoxic group that were treated with 249 mg/kg of the extract (group V) compared to the positive control group (group II).The result as presented in table III also shows that the level of AST, ALP, ALT, Total bilirubin and Direct bilirubin are significantly ($P < 0.05$) lower in hepatotoxic groups treated with 580mg/kg of extract (group VI) compared to the positive control group (group II). This suggests that both 249 mg/kg and 580 mg/kg of the extract may have hepatocurative effect. Comparison between hepatotoxic group treated with 249mg/kg of extract (group V) and hepatotoxic group treated with 580mg/kg of the extract (group VI) shows that the level of AST, ALT, ALP, direct and total bilirubin are significantly ($P < 0.05$) lower in group VI compared to group V. This may suggest that the 580 mg/kg of the extract has more hepatocurative effect than the 249 mg/kg of the extract.

Table III: Effect Of Oral Administration Of Aqueous Extract Of *Phoenix Dactylifera* On Biochemical Parameters In Hepatotoxic Group Treated With 249 Mg/Kg Of Extract (Group V) And Hepatotoxic Group Treated With 580 Mg/Kg Of The Extract (Group Vi)

GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TOTAL BIL (mg/dl)	DIRECT BIL (mg/dl)
GROUP I	36.36^{a} ± 1.17	44.75^{a} ± 1.19	78.55^{a} ± 1.20	0.95^{a} ± 0.04	0.50^{a} ± 0.07
GROUP II	105.23^{a} ± 2.74	93.22^{a} ± 1.31	114.53^{a} ± 3.75	1.97^{a} ± 0.05	1.26^{a} ± 0.18
GROUP V	90.26^{a} ± 2.24	87.81^{a} ± 1.69	98.39^{a} ± 1.31	1.47^{a} ± 0.18	1.07^{a} ± 0.13
GROUP VI	45.59^{a} ± 2.44	49.65^{a} ± 1.37	90.07^{a} ± 1.88	1.02^{a} ± 0.17	0.52^{a} ± 0.07

Values are expressed as mean ± SD
Values in the same column bearing the same superscript are significantly different at $P < 0.05$, n= 5

IV. DISCUSSION

The result of this research (in Table I) shows that administration of 100 mg/kg of CCl_4 subcutanously once to normal (healthy) rats induce hepatotoxicity after 48 hours. This is evident because of the significant ($P < 0.05$) increase in the level of serum AST, ALT, ALP, total bilirubin and direct bilirubin in group II (positive control) compared to group I (negative control). This is in consistence with the report of [11] who confirmed that high doses of CCl_4 (90 - 120mg/kg) can induce massive liver damage and may persist for a long period. The hepatotoxic effects of CCl_4 occurs when CCl_4 is metabolized by the cytochrome-P-450 systems in the liver into a highly reactive metabolite – trichloromethyl radical, $\cdot CCl_3$, which binds covalently to macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic recticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, thus initiating lipid peroxidation

[12]. Lipid peroxidative degradation is one of the principal causes of hepatotoxicity by CCl_4. This was confirmed in this research by the elevation in the serum level of AST, ALP and ALT in group II. These are all non plasma specific enzymes. These enzymes were reported to be higher in hepatotoxic rats than in normal rats when there is liver necrosis [13]. The concurrent elevation of serum AST together with ALP and ALT more likely indicates hepatotoxicity induced by CCl_4 [14]. Also, the increase in total and direct bilirubin level in the positive control group (group II) compared to the negative control group (group I) indicates the presence of hyperbilirubunaemia. These observations are all indicators of hepatic cell damage and are in accordance with the report of [15], [16].

The administration of different concentrations (249 mg/kg and 580 mg/kg) of aqueous extract of date fruit to healthy rats (Table II) shows no significant ($P > 0.05$) increase in the level of AST, ALT, ALP, total and direct bilirubin in group III and IV compared to group I. This indicates that aqueous extract of date fruit may have no harmful effect. The "harmless" effect of date fruit was already reported by [10].

Date fruit have been used extensively for traditional cure of illness in Arab peninsular [17]. In this research, the administration of 249mg/kg of aqueous extract of date fruit to hepatotoxic rats (Table III) resulted in significant ($P < 0.05$) decrease in the level of AST, ALT, ALP, direct bilirubin and total bilirubin. Also, the administration of 580 mg/kg of aqueous extract of date fruit to hepatotoxic rats (Table III) resulted in significant ($P < 0.05$) decrease in the level of AST, ALT, ALP, direct bilirubin and total bilirubin. All these results suggest that administration of either 249mg/kg or 580mg/kg of aqueous extract of date fruit may have hepatocurative effect. This is in accordance to the findings of [7]. The hepatocurative effect of date fruit could be due to the presence of antioxidants such as flavonoids and vitamin C [18]. Flavonoids are phenolic substances that have the ability to reduce free radical formation and scavenge free radicals. Most ingested flavonoids are extensively degraded to various phenolic acids, some of which still possess radical scavenging ability. Both the absorbed flavonoids and their metabolites may display an *in vivo* antioxidant activity [19]. It was also found that phenolic and polyphenolic compounds are very efficient scavengers of free radicals because of their molecular structures, which include an aromatic ring with hydroxyl groups containing mobile hydrogen [20]. Moreover, the action of phenolic compounds can be related to their capacity to reduce and chelate ferric ion, which catalyse lipid peroxidation [21].

V. CONCLUSION

The present research reveals that the various/different concentration of aqueous extract of date fruit (*Phoenix dactylifera)* used in this research may have hepatocurative effect.

CONFLICT OF INTEREST

There is no any conflict of interest between the authors in whatever form.

REFERENCES

[1] Kerry, H. C. and Janice, L. H. (2013). "Brunner Text book of medical-surgical nursing". Lippincott Williams and Wilkins.

[2] Ham, A. A. (1974) "A text book of Histology" 7th edition. JB Lippincot company,Philadelphia and Toronto.Pp 686.

[3] Cotran, R. S., Kumar, V., Fausto, N., Nelson, F., Robbins, S. L., Abbas, A. K. (2005). "Robbins and Cotran pathologic basis of disease" (7th ed.) St. Louis, M O: Elsevier Saunders. P878.

[4] Nelson, D. L. and Cox, M. M. (2005). "Lehninger Principle of Biochemistry". Fourth edition. W H Freeman and company, New York.

[5] Nwokediuko, S. C., Osuala, P. C., Uduma, U. V., Alaneme, A. K., Onwuka, C. C. and Mesigo, C. (2013) "Pattern of liver disease admission in Nigerian tertiary hospital". Nijer J Clin Pract 2013; 16:339-42.

[6] Nuhu, H. (2001). "Pharmacognosy in A.B.U" Graphic media production. Zaria.

[7] Al-qarawi, A. A., Mousa, H. M., Ali, B. H., Abdel-Rahman. H. and Almagy, S. (2004) "Protective effect of extracts from dates (Phoenix dactylifera L.) on carbon tetrachloride-induced hepatotoxicity in rats". International Journal of App/Rev Veterinary Medicine .Vol. 2, No. 3.

[8] El Hadrami, A., Daayf, F. and El Hadrami, I (2011). "Secondary metabolites of date palm" In: Jain, S. M., Alkhayri, J. M. and Johnson, D. V.

[9] Mohammed, D, A. and Al-okbi, S. Y. (2004) "Invivo evaluation of antioxidant and anti inflammatory activity of different extracts of date fruit in adjuvant arthritis". Polish Journal of food and Nutrition Sciences.Vol.13154, No 4.Pp 397-402.

[10] Agbon, A. N., Kwanashie, H. O. and Hamman, W. O (2013) "Evaluation of antidiarrheal effect of the aqueous fruit extract of Phoenix dactyliferaL . on the histology of the small intestine of wister rats". British Journal of pharmacology and toxicology. 4(3): 121-127.

[11] Alhassan, A. J., Sule, M .S., Hassan. J. A., Baba, B. A. and Aliyu, M. D. (2009) "Ideal hepatotoxicity model in rats using carbon tetrachloride (CCl_4)".Bayero Journal of pure and applied sciences, 2(2) :185-187.

[12] Recknegel, R. O and Glende, E. A. (1973) "CCl_4 hepatotoxicity: an example of lethal cleavage". CRC critical review toxicology, 2:263-297.

[13] Keith, G. T. and Robert, R. E. J. (2001). "Liver function In: Tietz fundamentals of clinical chemistry". 5th edition. W.B Saunders company. Pp 747-70.

[14] Nduka, N. (1999) "Clinical Biochemistry for students of pathology" Longman Nigeria Plc

[15] Venukumar, M. R and Latha, M. S (2002). "Hepatoprotective effect of the methanolic extract of Curculigo orchioides in CCl_4-treat male rats". Internationa Journal of Pharmacol. 34:269-75.

[16] Etuk, E. U., Agaie, B.M., Ladan, M. J. and Garba, I. (2009). "The modulatory effect of Cochspermum tinctoriuma rich aqueus root extract on liver damage induced by carbon tetrachloride in rats". African Journal of pharmacy and pharmacology.Vol 3 (4). Pp 151-157.

[17] Alshoaibi, Z., Amamary, M. A., Al-Habori, M. A., Al Zubair, A. S. and Abdelwahab, S. I (2012) "Invivo antioxidant and hepatocurative effect of palm date fruit (Phoenix dactylifera)". International Journal of pharmacology, 8 : 185-191.

[18] Faqir, M. A., Sardar, I. B., Ahmad, H. E., Muhammad, I. K. and Muhammad, S. A. (2012). "Phytochemical characteristics of date palm (Phoenix dactylifera) fruit extracts". Pakistan Journal of Food Science. 22(3):117-127.

[19] Pietta, P, G. (2000) "Flavonoids as antioxidants". J Nat Prod. 63(7):1035-42.

[20] Halliwell, B. (1994). "Free radicals and antioxidants:A personal view" Nutr. Res.52:253 - 265.

[21] Gazzani, G., Papetti, A. and Massolini, G. (1998) "Anti-and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment". Journal of Agric. And Food chemistry.46:4118 - 4122