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Synergistic Hepatoprotective Effect of Grape Juice with Date Palm Fruit Methanolic Extracts

A. H. Atta[1], K. Abo-EL-Sooud[1*], Sohair Saied Ahmed[2], Shereen Ibrahim[1] and Shimaa Zaher[2]

[1]Pharmacology department, Faculty of Veterinary Medicine, Cairo University
[2]Nutrition and Food science department, Faculty of Home Economics, Al-Azhar University

K. Abo-EL-Sooud[*] (Corresponding author)
Dept. of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza PO box 12211, Egypt

Tel: +201066756870 E-mail: kasooud@yahoo.com

Abstract

The present work was carried out to evaluate the hepatoprotective effect of grape juice, date palm fruit methanolic extracts (600 mg/kg b.wt.) and their combination (1:1 or 1:2) given orally for 21 day against CCl_4 intoxication in rats. There was no significant alteration in most of the hematological parameters tested. Their administration in CCl_4-intoxicated rats significantly ($P < 0.05$) decreased ALT and GGT activity in serum of rats as compared to rats treated with CCl_4 only. Oral administration of methanolic extract of grapes and date palm fruit (1: 1 or 1:2 w/w) significantly ($P < 0.05$) decreased ALT, AST and GGT activity in serum of rats as compared to rats treated with CCl_4 only. The tested extracts significantly ($P < 0.05$) decreased glucose, bilirubin, serum triglycerides and cholesterol levels as compared to rats treated with CCl_4 only. However, their administration significantly ($P < 0.05$) increased protein level in serum of rats as compared to rats treated with CCl_4 only. In conclusion the use of combination grape juice, date palm fruit methanolic extracts (1:2) caused marked hepatoprotective effect as confirmed by the histopathological examination.

Keywords: GC/MS, Grape, Date palm, Hepatoprotective, Synergism

1. Introduction

Vitis vinifera (grape) and *Phoenix dactylifera L.*, (date palm fruits), are valuable plants that grow in the Southwest Asia and North Africa. Both edible plants contain various nutrient elements, such as vitamins, minerals, carbohydrates, edible fibers and phytochemicals. Grapes and date fruits are significant components of the diet in the majority of the Arab countries with low cost. For Muslims, dates are of religious value and have been mentioned several times in the Quran. They are usually breaking their long day fasting with dates in the month of Ramadan (**Vayalil, 2002, Al-Farsi and Lee, 2008 and Baliga *et al.*, 2011**). Polyphenols are the most important phytochemicals in grape an date because they possess many biological activities and health-promoting

benefits **(Silva *et al.*, 1991 and Baliga *et al.*, 2011)**. The phenolic compounds mainly include anthocyanins, flavanols, flavonols, stilbenes (resveratrol), carotenoids and procyanidins flavonoids **(Cantos *et al.*, 2002 and Al-Farsi and Lee, 2008)**. These natural compounds are known to function as free radical scavenger, antioxidant, antimutagenic, anti-inflammatory, hepatoprotective and nephroprotective agents **(Takaeidi *et al.*, 2014)**. Liver dysfunction caused mostly due to exposure to toxic chemicals, certain drugs and environmental pollutants, has been on the increase for the past few decades. In many countries hepatopathy is increasingly being managed using herbal treatment. **(Mitra *et al.*, 1998).** Carbon tetrachloride (CCl_4) is widely used for experimental induction of liver damage **(Parola *et al.*, 1992)**. The principle cause of CCl_4 is induced hepatic damage in lipid peroxidation in adipose tissue and it is metabolised to trichloromethyl radical and trichloromethyl peroxy radical which are involved in pathogenesis of liver **(Recknagel *et al.*, 1989)** and decreased activities of antioxidant enzymes and generation of free radicals **(Poli, 1993)**. One of the documented health promoting activities of many fruits and vegetables is their ability to scavenge naturally produced free radicals and hence acting as antioxidants **(Bauer, 2002)**. The present stud was carried out to evaluate the effects of repeated daily oral administration of grapes and date palm fruits methanolic extracts on carbon tetrachloride–induced hepatotoxicity in rats.

2. Material and Methods

2.1 Plants

Two plants were used in this study; grape fruits (*Vitis vinifera L.)* and date palm (*Phoenix dactylifera L.)* fruit. The plants were identified by the Flora & Phyto-Taxonomy Researches, Horticultural Research Institute, Agricultural Research Centre, Dokki, Giza.

Voucher samples of the tested plants are kept in the Pharmacology Department, Faculty of Veterinary Medicine, Cairo University.

2.2 Preparation of the plant extracts

Two hundred grams of the plants (grapes and date palm fruit) were extracted by percolation several times with methanol 90% for several times till complete exhaustion. The solvent was then evaporated by using Rota-vapour apparatus at 40 ^{0}C till a semisolid methanolic extract was obtained. The semisolid extract was divided into small portions and kept refrigerated until used. Known grams from the obtained semisolid extract were dissolved using few drops of Tween 80 (suspending agent), then distilled water was added to prepare a solution with desired concentration.

2.3 Animals:

Hundred Swiss mice of average body weight of 24-28 g, fifty for each plant extract, were used for determination of the oral LD_{50}. Forty-two albino rats of both sexes (Sprague Dowley Strain) with average body weight of 150 -170 g. were used.

Animals were kept under hygienic conditions (20-26^{0}C room temperature and 60-70 % relative humidity) for two weeks for acclimatization. Animals were given *ad libitum* access to food and water and were handled according to the local rules and regulation of Experimental Animals, Cairo University.

2.4 Determination of LD_{50}:

LD_{50} of the studied extracts was determined as described by **Kerber, (1941)**. In this experiment, ten groups of five Swiss mice each were used for each plant extract. Mice in groups from one to ten received oral doses of methanolic extract of either grapes or date palm in doses from 500 to 5000 mg/kg .b.wt. Animals were placed in observation cages and symptoms of discomfort, mortality rate were recorded for 48 hour after oral administration.

2.5 Gas Chromatography–Mass Spectrometry (GC/MS) Analysis of methanolic extracts of grapes and date palm fruit:

GC/MS analysis of methanolic extracts of grapes and date palm were performed at Micro Analytical Center-Cairo University using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with a Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 ìMdf, composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 µl was employed (split ratio of 10:1). The Injector temperature were 250°C and Ion-source temperature were 280 ^{0}C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10^{0}C/min, to 200°C, then 5°C/min to 280°C, ending with a 10 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 50 to 1000 Da. Total GC running time was 10 minutes at scan speed 2000. Software adopted to handle mass spectra and chromatograms was Turbo Mass Ver5.2.0

2.6 Hepatoprotective effect:

2.6.1. Experimental Design:

Rats were assigned randomly into seven groups of six rats each treated as follows: Rats of groups I and II received orally, equal volume of distilled water and corn oil (2.5 ml/kg) respectively and kept as controls. Rats of group III were given CCl_4 (50% in corn oil) at oral dose of 2.5 ml/ kg b. wt. three times a week. Rats of group IV and V were treated orally with daily dose of either methanol extract of grapes or date palm 2 hours after CCl_4 administration, respectively. Rats of group VI and VII were treated orally with daily dose of a mixture of

methanol extract of grapes and date palm either 1:1 or 1:2, respectively 2 hours after CCl_4 administration. CCl_4 was given them three times per week for 21 days. Grape and/or date palm extracts were given daily at an oral dose of 600 mg/kg b.wt. for 21 consecutive days. Twenty four hours after the last dose, blood samples were collected for haematological and biochemical evaluations. All rats were then sacrificed after blood collection and livers specimens were collected for histopathological examinations.

2.6.2 Blood samples:

For studying the hematological changes, blood samples were taken from the medial canthus of the eye of each rat by clean dry heparinized capillary tubes into clean dry vials for estimation of erythrocytic, leucocytic and platelets counts, packed cell volume (PCV %) and haemoglobin content (Hb %). Another blood sample was taken from each rat, left at room temperature to clot and clear serum was separated by centrifugation and used for serum biochemical analysis. CBC was determined using Sysmex XT-2000iV Automated Hematology Analyzer Japan.

2.6.3 Estimation of enzymatic activity and serum constituents:

Serum aspartate aminotransferase **(AST)** and alanine aminotransferase **(ALT)** were measured according to the method of **Reitman and Frankel, (1957)**. Gamma glutamyltransferase **(GGT**) **(Szasz, 1969)**, Bilirubin **(Jendrassik 1938)** and glucose (**Trinder, 1969)** were also measured. Triglycerides were determined according to **Fassati and Prencipe 1982,** while total cholesterol was determined according to **Zlatkis *et al.*, 1953** and modified by **Fredrikson *et al.*, 1967.** Total protein was estimated by the method of **Henry, (1964).** All serum biochemical constituents were estimated using commercial kits from bioMérieux-Franc.

2.7 Histopathological Examination:

Liver of the sacrificed rats were taken from each rat and immersed in 10 % formalin solution. The specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Samples were then cleared in xylol, embedded in paraffin, sectioned (4-6 microns) and stained with Heamtoxylin and Eosin for histopathological examination according to the method of **(Sheehan and Harpchak, 1980)**.

2.8 Statistical analysis:

Statistical analysis was carried out by using one way ANOV followed by Duncan test and SPSS version 9.0. The difference of means at $P > 0.05$ is considered significant **(Snedecor and Cochran, 1989)**.

3. Results

3.1 Acute toxicity

There were no signs of discomfort or significant change in the general behavior of the animals. No deaths were recorded up to 48 hours at the highest administered dose (5g/kg) of the extracts.

3.2 Phytochemical

The methanolic extract of grapes was subjected GC/MS analysis. It was observed from Figure.1A and Table -1 that the methanolic extract of grapes contained 23 components. The major components were alpha-D-Glucopyranoside, O-alpha-D-Glucopyranosy1- (1. fwdarw. 3)-beta-D-fructofuranosy1, D-Glucose, 4-O- alpha-D-glucopyranosy1, sucrose, lactose and other carbohydrates. The methanolic extract of date palm fruit was subjected GC/MS analysis. It was observed from Figure 1B and Table 2 that the methanolic extract of date palm fruit contained 22 carbohydrate sugar components. The major components of date palm fruit are Sucrose, 1-Nitro- 1-deoxy -d- glycero -1- mannoheptitol , d-Glycero-d-ido-heptose, d-Glycero-d-galacto-heptose,Galacto-hept.ulose and d-Glycero-d-tallo-heptose.

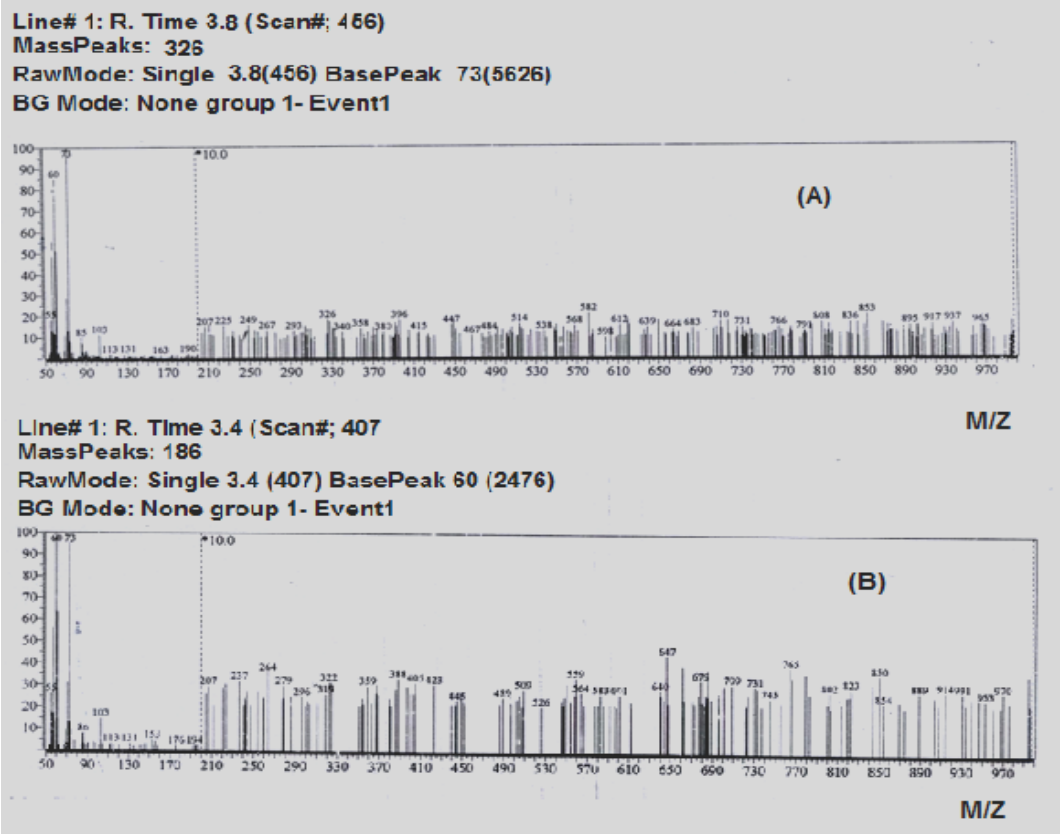

Fig. 1: Chromatogram obtained from the GC/MS with methanolic extract of grapes (A) and date palm (B).

Table 1: GC-MS analytical report for major phyto-constituents present in of methanolic extract of grapes

No.	RT	Name of the compound	Molecular Formula	MW	Base Peak
1	4506	alpha-D-Glucopyranoside , O-alpha-D-Glucopyranosy1- (1. fwdarw. 3)-beta-D-fructofuranosy1	$C_{18}H_{32}O_{16}$	504	73
2	3064	D-Glucose , 4-O- alpha-D-glucopyranosy1	$C_{12}H_{22}O_{11}$	342	73
3	3139	Sucrose	$C_{12}H_{22}O_{11}$	342	73
4	3064	Lactose	$C_{12}H_{22}O_{11}$	342	73
5	1536	2-Deoxy-D-galactose	$C_6H_{12}O_5$	164	31
6	1960	d-Glycero-d-ido-heptose	$C_7H_{14}O_7$	210	43
7	3064	alpha-D-Glucopyranose, 4-O- beta -D-galactopyranosy1	$C_{12}H_{22}O_{11}$	342	73
8	3131	beta -D-Glucopyranose, 4-O- beta -D-galactopyranosy1	$C_{12}H_{22}O_{11}$	342	73
9	1960	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$	210	43
10	3131	beta -D-Glucopyranose, 4-O- beta -D-galactopyranosy1	$C_{12}H_{22}O_{11}$	342	73
11	6530	Stevioside	$C_{38}H_{60}O_{18}$	804	60
12	3048	D-Glucose , , 6-O- alpha -D-galactopyranosy1	$C_{12}H_{22}O_{11}$	342	73
13	2937	2-Formy1-9-[beta -d- ribofuranosy1] hypoxanthine	$C_{11}H_{12}N_4O_6$	296	44
14	1988	Galacto-heptulose	$C_7H_{14}O_7$	210	43
15	1400	3,4-Altrosan	$C_6H_{10}O_5$	162	60
16	1960	d-Glycero-d-tallo-heptose	$C_7H_{14}O_7$	210	73
17	3091	2-[2-Hydroxyethy1] -9- [beta- ribofuranosy1] hypoxanthine	$C_{12}H_{16}N_4O_6$	312	43
18	1404	1,6-anhydro- beta -D-Glucopyranose (levoglucosan)	$C_6H_{10}O_5$	162	60
19	1678	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236	43
20	1988	D-Mannoheptulose	$C_7H_{14}O_7$	210	60
21	1353	alpha-D-Glucopyranoside, methy1 3,6-anhydro-	$C_7H_{12}O_5$	176	29
22	1698	d-Mannose	$C_6H_{12}O_6$	180	73
23	0	1- beta -d- Ribofuranosy1 -3- [5-tetraazolyl]-1, 2,4,-triazole	$C_8H_{11}N_7O_4$	269	57

Table 2: GC-MS analytical report for major phyto-constituents present in of methanolic extract of date palm fruit

No.	RT	Name of the compound	Molecular Formula	MW	Base Peak
1	1698	L-Mannose	$C_6H_{12}O_6$	180	73
2	1765	alpha-D-Glucose	$C_6H_{12}O_6$	180	73
3	1765	D-Mannopyranose	$C_6H_{12}O_6$	180	31
4	1765	D(+)-Talose	$C_6H_{12}O_6$	180	43
5	1960	d-Glycero-d-ido-heptose	$C_7H_{14}O_7$	210	31
6	1461	DL-Arabinose	C5H10O5	150	43
7	1960	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$	210	31
8	1726	1-Sorbose	$C_6H_{12}O_6$	180	31
9	1436	DL-Xylose	$C_5H_{10}O_5$	150	73
10	1436	L-Arabinose	$C_5H_{10}O_5$	150	73
11	1698	d-Mannose	$C_6H_{12}O_6$	180	73
12	3139	Sucrose	$C_{12}H_{22}O_{11}$	342	43
13	1988	Galacto-heptulose	$C_7H_{14}O_7$	210	73
14	2153	1-Nitro- 1-deoxy -d- glycero -1- mannoheptitol	$C_7H_{15}NO_8$	241	73
15	1505	D(+)-Ribonic acid gamma -lactone	$C_5H_8O_5$	148	73
16	1765	beta -D-Glucopyranose	$C_6H_{12}O_6$	180	73
17	1726	Fructose	$C_6H_{12}O_6$	180	73
18	1960	d-Glycero-d-tallo-heptose	$C_7H_{14}O_7$	210	73
19	1436	Xylose	$C_5H_{10}O_5$	150	73
20	1765	L-Glucose	$C_6H_{12}O_6$	180	73
21	1400	3,4-Altrosan	$C_6H_{10}O_5$	162	60
22	1436	2,3,4,5-Tetrahydroxypentanal	$C_5H_{10}O_5$	150	73

3.3 Effect on Hemogram and Biochemical Constituents:

Carbon tetrachloride partially decreased the number of RBCs, hemoglobin and HCT and significantly increased the RDW. Administration of methanol extract of grapes and/or Date palm normalized these changes (table 1). Moreover, CCL_4 significantly increased WBCs, lymphocytes and monocytes count. On the other hand administration of grape or date palm methanol extract normalized these values but significantly increased the granulocyte counts (Fig. 1). Minimal changes in platelet parameters except LPCR which was significantly increased by CCl_4 and normalized by methanol extract of grapes and /or date palm (Fig. 2) Oral administration in CCl_4-intoxicated rats significantly ($P < 0.05$) increased AST, ALT and GGT in serum of rats. Methanolic extract of grapes and date palm fruit (1 : 1 w/w , 1: 2 w/w) in CCl_4-intoxicated rats significantly ($P < 0.05$) decreased ALT, AST and GGT activity in serum of rats as compared to rats treated with CCl_4 only (Table 2). Oral administration in CCl_4-intoxicated rats significantly ($P < 0.05$) increased serum glucose, bilirubin, total protein, triglycerides and cholesterol levels. Oral administration of methanolic extract of grapes and date palm fruit alone or in combination into CCl_4-intoxicated rats significantly ($P < 0.05$) decreased glucose, bilirubin, triglycerides and cholesterol but significantly ($P < 0.05$) increased protein levels in serum of rats as compared to rats treated with CCl_4 only (Table 3).

3.4 Histopathological findings:

Liver of CCl_4- intoxicated rats showed fatty change of hepatocytes and cellular hyperplasia (Figure 1b) as compared to liver of normal rats (Figure 1a).. Liver of CCl_4- intoxicated rats treated with grapes and date palm alone showed fatty change of hepatocytes only (Figure 1 c and d). Liver of CCl_4 - intoxicated rats received grapes and date palm in combination showed improved architecture of hepatocytes (Figure 1 e & f).

4. Discussion

The present results markedly demonstrated that Grapes and / or date palm methanol extract normalized the hemopoietic changes induced by CCl_4 intoxication. They improved significantly the number of RBCs, hemoglobin and HCT. They also normalized the WBCs, lymphocytes and monocytes count which were elevated by CCl_4. **Onuh *et al.*, 2012** found that oral administration of methanolic extract of *P. dactylifera* daily for 112 days revealed dosage dependent significant increase in absolute values of red blood cells (RBC), haemoglobin (Hb), haemtatocrit (PCV), reticulocytes and platelet counts when compared with the controls. On the other hand, **Yamakoshi *et al.*, (2002)** found that administrations of grape extract for 90 days did not alter hematological parameters. The improved hematological parameters by methanol extract of grape and or date palm could be attributed to a hemopietic effect (**Onuh *et al.*, 2012)** as they are a great polyphenol source, which contains catechin, epicatechin, and procyanidins (B(1), B(2), B(3), and B(4) and posses a large amounts of elements such as potassium, calcium, magnesium, and iron which are important for hemopoiesis **(Dani *et al.*, 2009)**

CCl_4 produces oxidative damages and leakages of ALT, AST, GGT, total bilirubin in serum levels and decrease in total protein. Estimating the activities of serum marker enzymes like AST, ALT and GGT can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. The mechanism by which CCl4 causes liver damage involves the biotransformation of CCl_4 by the cytochrome P-450 enzyme system to the toxic trichloromethyl free radical (CCl3●), and then transforming this free radical into a more reactive trichloromethyl peroxyl radical (CCl3O2●), which causes lipid peroxidation, disrupts Ca2+ homeostasis, and eventually kills cells **(McCay *et al.*, 1984; Recknagel *et al.*, 1989)**. Moreover, The decline in protein content may be due to defects in protein biosynthesis as well as disruption and disassociation of polyribosomes from endoplasmic reticulum following administration of CCl_4 **(Clawson, 1989)**. Therefore, the reduction in levels of ALT, AST, GGT, total bilirubin and increased in total protein by the grapes and date palm fruit extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by $CC1_4$ **(Frank *et al.* 2012)**. The tendency of these enzymes to return towards a near normal level in treated rats is a clear indication of hepatoprotective effect of methanol extracts of grapes and date palm fruits. Moreover, the hepatoprotective effect of the tested extracts could be attributed to their antioxidant effect (**Opie and Lecour, 2007, Gatz and Wiesmuller, 2008, Qusti, *et al.*, 2010)** due to their content of strong antioxidant standards; vitamins C and E and the high levels of polyphenols like resveratrol (a polyphenol antioxidant).

Oral administration of methanolic extract of grapes and date palm fruit in CCl_4-intoxicated rats decreased the CCl_4 - elevated glucose. This effect is similar to their effect on streptozotocin-induced diabetic rats **(Orhan *et al.*, 2006)** who attributed the effect to the grape seed content of procyanidins. In another study, **El-Alfy *et al.*, 2005** found that pretreatment with orally administered proanthocyanidins from red grape seeds significantly inhibited the rise in blood glucose levels after alloxan injection compared with control rats. Repeated daily administration of the proanthocyanidin preparation for 72 h increased insulin levels in the blood back to control levels. The proanthocyanidins decreased lipid peroxidation and increased pancreatic glutathione levels. These data indicate that the grape seed proanthocyanidins protected b-cell function and suggest a protective effect against generation of damaging reactive oxygen species. In another study, **El-Alfy *et al.*, 2005** proanthocyanidins administration were reported to inhibit the rise in blood glucose levels after alloxan administration, to increase insulin levels in the blood back to control levels and to decrease lipid peroxidation and increased pancreatic glutathione levels. Those authors suggest that proanthocyanidins of grape seed protected β-cell function against generation of damaging reactive oxygen species. The mechanism of action of the dates could be similar to that of hypoglycaemic sulphonylureas, as closure of K^+–ATP (adenosine 5-triphosphate) channels. This result in membrane depolarization and increased Ca^{2+} influx, this will be an initial step in insulin secretion **(Mard *et al.*, 2010).** There is evidence to support dates benefits when mixed with meals in terms of glycaemic control **(Gilbertson *et al.*, 2001)**. The fruit and leaves of the date are used as a popular medicine in the United States and southwest Iran to reduce blood glucose level in diabetes **(Rock *et al.*, 2009,**

Aryangat and Gerich, 2010). Treatment with *Phoenix dactylifera* (date palm) leaf extract (PDE) had great raise concentration of plasma insulin in alloxan-induced diabetic rats. Composition of various types of dates alone or in mixed meals with plain yoghurt may be of benefit in glycaemic control in diabetic patients (**Miller *et al.*, 2003).**

The co-administration of methanolic extract of grapes and date palm fruit (1: 2 w/w) significantly decreased triglycerides and cholesterol as compared to their levels in oil and CCl_4-intoxicated rats. Nearly similar observations were reported by **Zunino *et al.*, 2014** who suggested that dietary grapes may decrease atherogenic lipid fractions in obese individuals and **Mildner-Szkudlarz and Bajerska (2013)** who reported the effect lowering effect of two different forms of grape by-products, namely dried powdered skins (PGP) and freeze-dried extract (EGP) on total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) levels and preventing visceral fat accumulation and increasing high-density lipoprotein cholesterol in hypercholestremic rats. There was no available literature concerning the effect of date palm fruit on blood total lipids, triglycerides or cholesterol level. However, studies showed that feeding rats with diet containing defatted date seed flour at 1.5%, 2.5% and at 5.2% concentration decreased plasma triglycerides, total cholesterol and low density lipoprotein **(Al-Maiman, 2005). Rock *et al.*, (2009)** investigated that after 4 weeks Medjool or Hallawi dates consumption, the VLDL-cholesterol levels tended to be reduced (by 8 or 15%, respectively, with value of $0.1 > p > 0.05$). As well as in human, the dietary fiber feeding reduces blood cholesterol concentration. The findings of these studies suggested that diet based on date seed fiber had a good major source of dietary fiber (**Evans *et al.* 1992 and Kattak, 2002).** Date Plant leaves extracts could have a protective effect against hyperlipidemia through improvement of lipid profile **(Abuelgassim, 2010).** Moreover, palm oil from tree of *Elaeis guineesis*, was proved to have an anti-angiogenesis, cholesterol inhibition, brain development and neuro-protective properties, antioxidative defense mechanisms, provitamin A activity and anti-diabetes **(Loganathan *et al.*, 2010)**. The mechanism by which the date palm fruit extract induces its hepato-protective activity against oxidative damage caused by CCl_4 is not certain. However, it is possible that polyphenolic compounds (flavonoids, anthocyanins and phenolic acids), and trace elements (selenium, copper, zinc and manganese), in addition to vitamin C present in the date palm fruit **(Al-Farsi *et al.*, 2005)** are the responsible compounds for this protection. The improved histopathological picture of the liver of rats treated with grapes and / or date palm extracts confirmed the reported improvement in the hemogram, improved serum biochemical constituents and hepatoprotective effect. In conclusion methanol extract of grapes and date palm induced a promising hepatoprotective effect against CCl_4 induced hepatic damage. Combination of grapes and Date palm fruit methanol (1:2 w/w) gave the best results.

5. References

Abuelgassim, A.O. (2010). Effect of flax seeds and date palm leaves extracts on serum concentrations of glucose and lipids in alloxan diabetic rats. Pak. J. Biol. Sci., 13:1141-1145.

Al-Farsi, M.; Alasalvar, C.; Morris, A.; Baron, M. and Shahidi, F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera L.*) varieties grown in Oman. J. Agric. Food Chem., 53(19):7592-7599.

Al-Farsi, M.A. and Lee, C.Y. (2008). Nutritional and functional properties of dates: a review. Crit. Rev. Food Sci. Nutr., 48(10):877-887.

Al-Maiman, S. (2005). Effect of date palm (*Phoenix dactylifera*) seed fibers on plasma lipids in rats. J. Kig saud Univ., Vol. Agric. Sci., 2: 117-123.

Aryangat, A.V. and Gerich, J.E. (2010). Type 2 diabetes: postprandial hyperglycemia and increased cardiovascular risk. Vasc. Health Risk Manag., 6:145-155.

Baliga, M.S.; Baliga, B.R.V.; Kandathil, S.M.; Bhat, H.P. and Vayalil, P.K. (2011). A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera L.*). Food Res. Int.; 44(7):1812–1822.

Bauer, J. (2002): The complete Idiot's guide to total nutrition. Alpha books, 76-78, 27.

Cantos, E.; Espin, J.C. and Tomas-Barberan, F.A. (2002). Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. J. Agric. Food Chem., 50: 5691–5696.

Clawson, G.A. (1989). Mechanisms of carbon tetrachloride hepatotoxicity. Pathol. Immunopathol. Res.,8: 104-112.

Dani, C.1.; Oliboni, L.S.; Vanderlinde, R.; Pra, D.; Dias, J.F.; Yoneama, M.L.; Bonatto, D.; Salvador, M.; Henriques, J.A. (2009) Antioxidant activity and phenolic and mineral content of rose grape juice. J Med Food. Feb;12(1):188-92.

El-Alfy, A.T.; Ahmed, A.A.E. and Fatani, A.J. (2005). Protective effect of red grape seeds proanthocyanidins against induction of diabetes by alloxan in rats. Pharmacol. Res.,52:264–270.

Evans, A. J.; Hood, R.L.; Oakenfull, D.G. and Sidhu, G.S. (1992). Relationship between structure and function of dietary fibre: a comparative study of the effects of three galactomannans on cholesterol metabolism in the rat. Br. J. Nutr., 68: 217-229.

Fassati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28: 2077.

Frank, P.R.; Suresh, V.; Arunachalam, G.; Kanthlal, S.K. and Ziaudheen, V.M. (2012). Evaluation of hepatoprotective effect of *Adiantum incisum* forsk leaf extract against CCl_4 induced hepatotoxicity in rats. IRJP, 3 (3): 230-234.

Fredrikson, D. S.; Levi, R. L. and Less, R. S. (1967). Determination of cholesterol. N. Engl. J. Med., 276: 148 – 156.

Gatz, S.A. and Wiesmuller, L. (2008). Take a break-resveratrol in action on DNA. Carcinogenesis, 29(2): 321–332.

Gilbertson, H. R. Brand Miller, J.C.; Thorburn, A.W.; Evans, S.; Chondros, P. and Werther, G.A. (2001). The effect of flexible low glycaemic index dietary advice versus measured carbohydrate exchange diets on glycaemic control in children with type 1 diabetes. Diabetes Care, 24: 1137-1143.

Henry, R. J. (1964). Clinical Chemistry. Colorimetric determination of total protein. In: Harper and Row Publ., New York, p 181.

Jendrassik, L. (1938). Determination of bilirubin. Biochem. J. 7297: 7281.

Kattak, M. (2002). Physiologically effect of dietary complex carbohydrates and its metabolite roll in some desease. Pakistan Journal of Nutrition, 4:161-168.

Kerber G (1941): Pharmakologische Methoden Zur Affindung Von Arzneimittem Und Gifter Und Analyse Ihrer Wirkungsweise Dr. Med. Leopold Ther. Wissenschaftjihe Verlage Geesegesellschaft, M.B.H.

Loganathan, R. Jr .; Selvaduray, K.R.; Nesaretnam, K. and Radhakrishnan, A.K. (2010): Health promoting effects of phyto-nutrients found in palm oil. Malays. J. Nutr., 16(2):309-322.

Mard, S.A.; Jalalvand, K.; Jafarinejad, M.; Balochi, H. and Naseri, M.K. (2010). Evaluation of the antidiabetic and antilipaemic activities of the hydroalcoholic extract of *Phoenix dactylifera* palm leaves and its fractions in alloxan-induced diabetic rats. Malays. J. Med. Sci.,17: 4-13.

McCay, P. B.; Lai, E. K.; Poyer, J. L.; DuBose, C. M.; Janzen, E. G.(1984). Oxygen and carbon-centered free radical formation during carbon tetrachloride metabolism. J Biol Chem. 1984;259:2135–2143.

Mildner-Szkudlarz, S. and Bajerska, J. (2013): Protective effect of grape by-product-fortified breads against cholesterol/cholic acid diet-induced hypercholesterolaemia in rats. J. Sci. Food Agric., 93(13):3271-3278.

Miller, C.J.; Dunn, E.V and Hashim, I.B. (2003). The glycaemic index of dates and date/yoghurt mixed meals. Are dates 'the candy that grows on trees'? Eur. J. Clin. Nutr., 57: 427-430.

Mitra, S.K.; Venkataranganna, M.V.; Sundaram, R.; Gopumadhavan, S. (1998). Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. J Ethnopharmacol.;63(3):181-6.

Onuh, S.N.; Ukaejiofo, E.O.; Achukwu, P.U.; Ufelle, S.A.; Okwuosa, C.N. and Chukwuka, C.J. (2012). Haemopoietic activity and effect of crude fruit extract of *Phoenix dactylifera* on peripheral blood parameters. Int. J. Biol. Med. Res.; 3(2): 1720-1723.

Opie, L.H. and Lecour, S. (2007). The red wine hypothesis: from concepts to protective signaling molecules. European Heart Journal, 28(14): 1683–1693.

Orhan, N.; Aslan, M.; Orhan, D.D.; Ergun, F. and Yesilada E. (2006). *In-vivo* assessment of antdiabetic and antioxidant activities of grapevine leaves (*Vitis vinifera*) in diabetic rats. J. Ethnopharmacol.;108: 280–286.

Parola, M.; Leonarduzz, G.; Biasi, F.; Albono, M.; Biocca, G. and Polic, Dianzani, M.U. (1992). Vitamin E dietary supplementation. Protects against CCl_4 induced chronic liver damage and cirrhosis. Hepatology, 16:1014-1021.

Poli, G. (1993). Liver damage due to free radicals. Br. Med. bull. 49:604-620.

Qusti, S. Y.; Abo-khatwa, A. N. and Bin Lahwa, M. A. (2010). Screening of antioxidant activity and phenolic content of selected food items cited in the holly Quran. E. Journal of Biological Sciences, 2(1): 40-51.

Recknagel, R.D.; Glende, E.A.; Dolak, J.A. and Waller, S. (1989). Mechanism of carbon tetrachloride toxicity. Pharmacol. Ther., 43:139-154.

Reitman, S. M. D. and Frankel, S. (1957). A colorimeter method for determination of serum glutamic oxaloacetic acid glutamic pyruvic acid transferases. Am. J. Clin. Pathol. , 28:56-63.

Rock, W.; Rosenblat, M. and Borochov-Neori, H. (2009). Effects of date (*Phoenix dactylifera L*., Medjool or Hallawi Variety) consumption by healthy subjects on serum glucose and lipid levels and on serum oxidative status: a pilot study. J. Agric. Food Chem., 57: 8010-8017.

Sheehan, D.C. and Hrapchak, B.B. (1980). Theory and Practice of Histotechnology. 2nd Ed. The C.V. Mosby company, St. Louis, 205, London, UK.

Silva, R.C.; Rigaud, J.; Cheynier, V. and Chemina, A. (1991). Procyanidin dimers and trimers from grape seeds. Phytochemistry, 30: 1259–1264.

Snedecor, George W. and Cochran, William G. (1989). Statistical Methods, Eighth Edition, Iowa State University Press.

Szasz, G. (1969) A kinetic photometric method for serum gamma glutamyl transferase. Clin. Chem., 15:124–136.

Takaeidi, M.R.; Jahangiri, A.; Khodayar, M.J.; Siahpoosh, A.; Yaghooti, H.; Rezaei, S.; Salecheh, M. and Mansourzadeh, Z. (2014). The effect of date seed (*Phoenix dactylifera*) extract on paraoxonase and arylesterase activities in hypercholesterolemic rats. Jundishapur. J. Nat. Pharm. Prod., 9 (1):30-34.

Trinder, P. (1969). A Rapid Method for the Determination of glucose in Serum Analyst, 76: 596-599.

Vayalil, P.K. (2002). Antioxidant and antimutagenic properties of aqueous extract of date fruit (*Phoenix dactylifera L. Arecaceae*). J. Agric. Food Chem., 50(3):610-617.

Yamakoshi, J.; Saito, M.; Kataoka, S. and Kikuchi, M. (2002). Safety evaluation of proanthocyanidin-rich extract from grape seeds. Food Chem Toxicol.;40 (5):599-607.

Zlatkis, A.; Zak, B. and Boyle, A. J. (1953). A new method for direct determination of serum cholesterol. J. Lab. Chem. Med., 41: 486 – 492.

Zunino, S.J.; Peerson, J.M.; Freytag, T.L.; Breksa, A.P.; Bonnel, E.L.; Woodhouse, L.R.1. and Storms, D.H.1.(2014). Dietary grape powder increases IL-1β and IL-6 production by lipopolysaccharide-activated monocytes and reduces plasma concentrations of large LDL and large LDL-cholesterol particles in obese humans. J. Nutr., 112(3):369-380.

Table 3: Effect of methanol extract of Grapes (Gr) and Date palm (DPF) alone or in combination on blood indices in CCl_4-intoxicated rats (mean ± SD, n=6).

Groups	RBC (X 10^6 /µl)	RDW %	HGB g/dL	HCT %
Normal control	7.60±0.6^{ab}	12.62±0.6a	15.60±0.7^{b}	42.68±2.2^{c}
Corn oil	7.19±0.6^{ab}	14.71±1.2ab	14.22± 1.3^{ab}	39.24±3.5^{abc}
CCl4	6.99±1.1^{a}	17.93±2.6c	13.20± 1.8^{a}	37.43±4.8^{ab}
Gr + CCl_4	7.97±0.4^{b}	17.08±3.6bc	14.63±1.1^{ab}	41.18±3.5^{bc}
DPF+ CCl_4	7.54±0.5^{ab}	16.73±1.2bc	14.12± 1.^{ab}	39.48±2.7^{abc}
Gr + DPF+ CCl_4(1:1)	7.44±0.6^{ab}	16.25±2.0bc	13.95± 1.0^{a}	38.73±3.4^{abc}
Gr + DPF+ CCl_4(1:2)	7.39±0.2^{ab}	15.78±1.7^{bc}	13.65± 0.8^{a}	36.85±2.8^{a}

RBC: Red Blood Corpuscles, RDW: Red Cell Distribution Width, HGB: Haemoglobin, HCT: Haematocrite. Means with different superscript letters are significantly different at P ≤ 0.05

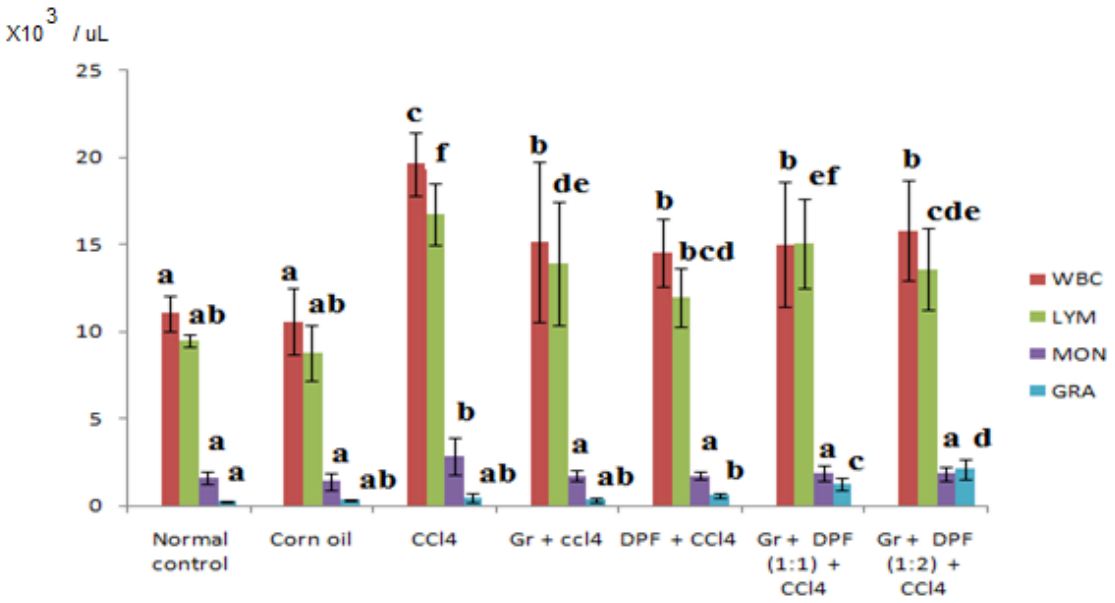

Fig. 3: Effect of methanol extract of Grapes (Gr) and Date palm (DPF) alone or in combination on WBCs and differential counts in CCl4-intoxicated rats (mean ± SD, n=6). WBC :Total leucocytes count , LYM : lymphocytes, MON : Monocytes, GRA : Granulocytes. Means with different superscript letters are significantly different at P ≤ 0.05

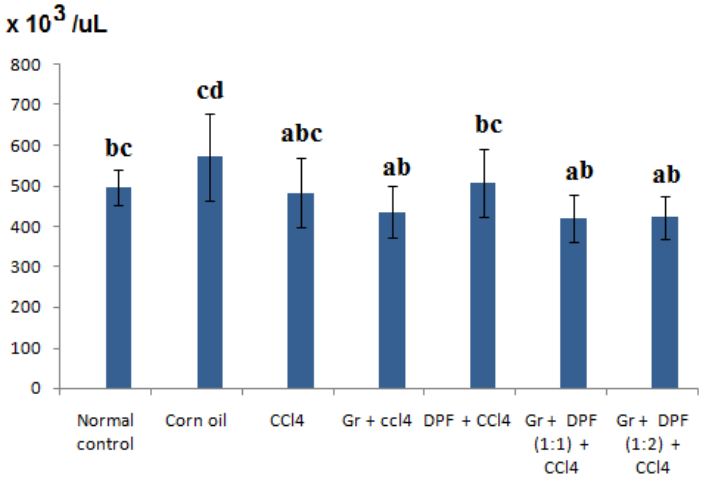

Fig. 4: Effect of methanol extract of Grapes (Gr) and Date palm fruit (DPF) alone or in combination on and total PLT in normal and CCl4-intoxicated rats (mean ± SD, n=6). PLT: Platelets. Means with different superscript letters are significantly different at $P \leq 0.05$

Table 4: Effect of methanolic extract of grapes (Gr) and date palm fruit (DPF) alone or in combination on serum ALT, AST and GGT level in normal and CCl_4-intoxicated rats (mean ± SD, n=6).

Groups	ALT U/L	AST U/L	GGT U/L
Normal control	28.08±4.89c	18.07±2.28^{a}	4.16±0.71^{ab}
Corn oil	16.34±0.37a	18.05±1.75^{a}	3.81±0.15^{a}
CCl_4	65.19±8.14e	44.58±0.75^{e}	14.45±0.47^{g}
Gr + CCl_4	26.54±0.90c	46.48±1.93^{e}	8.24±0.31^{d}
DPF + CCl_4	39.31±6.67d	32.73±1.73^{c}	11.25±0.90^{f}
Gr + DPF + CCl_4(1:1)	41.78±10.52d	36.40±2.94c	9.41±1.20^{e}
Gr + DPF + CCl_4(1:2)	22.02±4.35abc	24.52±5.24^{b}	7.44±0.63^{d}

Means with different superscript letters are significantly different at $P \leq 0.05$

Table 5: Effect of methanolic extract of grapes (Gr) and date palm fruit (DPF) alone or in combination on serum bilirubin, glucose, protein, Triglycerides and Cholesterol level in normal and CCl4-intoxicated rats (mean ± SD, n=6)

Groups	Bilirubin mg/dl	Glucose mg/dl	Protein g/dL	Triglycerides mg/dL	Cholesterol mg/dL
Normal control	0.43 ± 0.0^{a}	113.70 ± 9.7^{d}	10.25 ± 0.4^{de}	151.14 ± 6.2^{a}	93.93 ± 10.2^{b}
Corn oil	0.44 ± 0.1^{a}	116.87 ± 8.6^{d}	8.42 ± 0.8^{bc}	190.00 ± 10.8^{b}	93.90 ± 8.5^{b}
CCl_4	1.10 ± 0.2^{f}	131.24 ± 4.6^{e}	6.73 ± 0.5^{a}	265.29 ± 13.3^{d}	159.18 ± 19.5^{d}
Gr + CCl_4	0.83 ± 0.1^{de}	93.94 ± 7.3^{abc}	7.51 ± 1.3^{ab}	155.70 ± 13.1^{a}	75.79 ± 6.7^{a}
DPF + CCl_4	0.93 ± 0.0^{e}	90.25 ± 6.9^{abc}	8.45 ± 0.4^{bc}	212.82 ± 8.9^{c}	118.96 ± 11.5^{c}
Gr + DPF (1:1) + CCl_4	0.71 ± 0.1^{cd}	90.75 ± 3.8^{abc}	9.59 ± 1.1^{cd}	209.62 ± 5.1^{c}	104.40 ± 8.7^{b}
Gr + DPF (1:2) + CCl_4	0.64 ± 0.1^{bc}	84.14 ± 6.9^{a}	11.63 ± 2.5^{e}	164.47 ± 3.0^{a}	95.32 ± 5.3^{b}

Means with different superscript letters are significantly different at $P \leq 0.05$

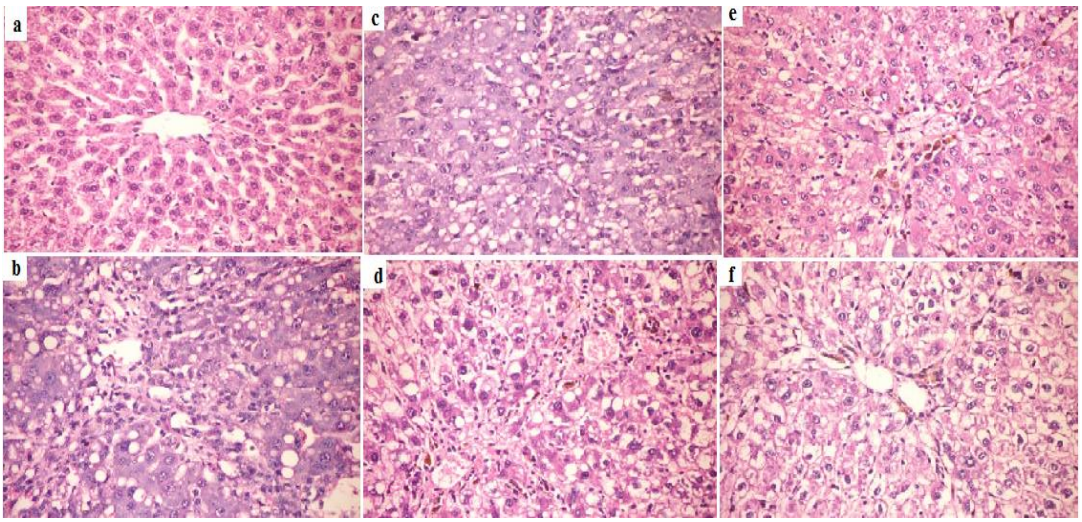

Figure 5: **a** : Liver of normal control rats **b** : Liver of CCl_4 =intoxicated rat showing fatty change of hepatocytes and cells hyperplasia; **c** : Liver CCl_4=intoxicated rat treated with grape extract showing fatty change of hepatocytes.; **d** : Liver of CCl_4 =intoxicated rat treated with date palm showing fatty change of hepatocytes and oval cells proliferation; **e**: Liver of CCl_4 =intoxicated rat treated with grapes and date palm (1:1) showing fatty change of hepatocytes and slight hyperplasia of oval cells; **f**. Liver of CCl_4 =intoxicated rat treated with grapes and date palm (1:2) showing vacuolar degeneration of hepatocytes (H & E, X400).