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In Vitro and *In Vivo* Antioxidant Potential of Hydromethanolic Extract of *Phoenix dactylifera* Fruits

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Abstract

The present study was aimed at investigating the antioxidant activities of the hydromethanolic extract of *Phoenix dactylifera* (HEPD) fruit (Arecaceae). The antioxidant activities of extract have been evaluated by using a range of *in vitro* assays and *in vivo* hepatoprotective model. In case of *in vitro* studies the IC_{50} values were found to be 160, 1400, 1115, 1050 µg/ml in DPPH, nitric oxide, superoxide, hydroxyl radical scavenging assays, respectively. In case of *in vivo* studies the levels of liver enzymatic, non-enzymatic systems [serum glutamate oxalo-acetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphatase (ALP), total bilirubin, total protein, catalase (CAT), glutathione (GSH), superoxide dismutase (SOD)] and lipid peroxidation (LPO) were restored towards the normal value in HEPD treated carbon tetrachloride intoxicated rats. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic (pyrocatechol and gallic acid contents are 6.2 and 2.906 µg/mg, respectively), vitamin C (ascorbic acid content is 0.66 µg/mg) and flavonoid compounds (4.79 µg/mg) present in HEPD. The results obtained in the present study indicate that the *Phoenix dactylifera* fruit is a potential source of natural antioxidant.

Keywords: *Phoenix dactylifera*; Antioxidant, Flavonoid; Total phenolic content; Vitamin C.

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1. Introduction

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging etc [1]. A free radical is defined as any atom or molecule possessing unpaired electrons. Antioxidants are agents which scavenge the free radicals and prevent the damage caused by reactive oxygen species (ROS), reactive nitrogen species (RNS). ROS is composed of superoxide anion ($O2^{\cdot}$), hydroxyl ($OH^{\cdot}$), hydroperoxyl ($OOH^{\cdot}$), peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$) radicals non free radicals are

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hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3) singlet oxygen ($^1O_2$). RNS are mainly nitric oxide ($NO^{\cdot}$), peroxynitrite ($ONOO^{\cdot}$) nitrogen dioxide (NO2). Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells prevent damage to lipids, proteins, enzymes, carbohydrates DNA. Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously include superoxide dismutase, catalase, glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases. There are some synthetic antioxidant compounds such as butylated hydroxytoluene, butylated hydroxyanisole tertiary butylhydroquinone which are commonly used in processed foods. However, it has been suggested that these compounds have shown toxic effects like liver damage mutagenesis. Flavonoids other phenolic compounds of plant origin have been reported as scavengers of free radicals. Hence, nowadays search for natural antioxidant source is gaining much importance.

The Date Palm (*Phoenix dactylifera*) [2] is extensively cultivated for its edible fruit. Dates have a high tannin content are used medicinally as a detersive [3] (having cleansing power) astringent in intestinal troubles. As an infusion, decoction, syrup, or paste, dates may be administered for sore throat, colds, bronchial catarrh, taken to relieve fever number of other complaints. One traditional belief is that it can counteract alcohol intoxication. The seed powder is also used in some traditional medicines. A gum that exudes from the wounded trunk is employed in India for treating diarrhea genito-urinary ailments. The roots are used against toothache. The pollen yields an estrogenic principle, estrone, has a gonadotropic effect on young rats.

The objective of the present study was to investigate the antioxidant activity of the HEPD fruits using different *in vitro* and *in vivo* carbon tetrachloride induced hepatoprotective models as well as determination of total phenolic, flavonoid and ascorbic acid content in order to evaluate a relationship between the antioxidant activity and the phytochemical constituents.

2. Materials Methods

2.1. Chemicals

1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Chemicals, USA. Nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), sodium nitroprusside, napthyl ethylene diamine dihydrochloride, ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ethylene diamine teta acetic acid (EDTA), DTNB[5,5'-dithiobis-(2-nitrobenzoic acid)], sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) butylated hydroxyl anisole (BHA), deoxyribose, potassium ferricyanide [$K_3Fe(CN)_6$], Folin-Ciocalteu's phenol reagent

(FCR), aluminium trichloride ($AlCl_3$), vitamin C, potassium iodide, iodine were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

2.2. Extract

Hydromethanolic extract of fruits were used in the present study. The fresh ripe fruits were collected in the month of April-May, 2008 from Jadavpur, West Bengal. Fruits were washed properly with distilled water and the edible portion is separated from the seeds of the fruits. Then the edible portions of the fruits were extracted by using hydromethanolic solution (20% methanol in water) in mixi-grinder. After that the extracted juice were filtered by using a clean cloth. The extract is concentrated first in Rotary vacuum evaporator and then on water bath. The concentrated extract is then kept in freeze for future use.

3. Experimental Procedure

All the following experiments 3.1-3.5 were repeated three times and the results averaged.

3.1. *In vitro* antioxidant activity

3.1.1. *DPPH radical scavenging activity*

DPPH radical scavenging activity was measured using the method of Cotelle *et al.* [4] with some modifications. 3 ml of reaction mixture containing 0.2 ml of DPPH (100 μM in methanol) 2.8 ml of test solution, at various concentrations (5, 10, 20, 40, 80, 160 320 μg/ml) of the extract fractions was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm using Beckman model DU-40 spectrophotometer. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation:

$$\text{Percentage inhibition} = (1 - \text{absorbance of test/absorbance of control}) \times 100 \quad (1)$$

3.1.2. *Nitric oxide*

In aqueous solution at physiological pH, sodium nitroprusside generates nitric oxide, which interacts with oxygen to produce nitrite ions [5], which can be measured by the Griess reaction. 1ml of 10 mM sodium nitroprusside was mixed with 1 ml of test solution of different concentrations (80, 160, 320, 500, 800, 1000 μg/ml) in phosphate buffer (pH 7.4) and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 1 ml was taken out 1 ml of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid 0.1% naphthyl ethylene diamine dihydrochloride) was added to it. Absorbance of the chromophore formed by the diazotization of nitrite with sulfanilamide subsequent

coupling with naphthyl ethylene diamine dihydrochloride was read at 546 nm percentage inhibition was calculated by comparing the results of the test with those of the control using Eq. (1).

3.1.3. *Hydroxyl radical scavenging activity*

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al.* [6]. Stock solutions of EDTA (1mM), $FeCl_3$(10mM), ascorbic acid (1mM), H_2O_2 (10mM), deoxyribose (10mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1 ml EDTA, 0.01 ml of $Fecl_3$, 0.1 ml H_2O_2, 0.36 ml of deoxyribose, 1.0 ml of the extract of different concentration (200, 400. 800, 1000, 1200 mg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, PH-7.4), 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37˚C for 1 hr. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA 1.0 ml of 0.5% TBA (in 0.025 M NaoH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using Eq. (1).

3.1.4. *Superoxide radical scavenging activity*

Superoxide anion scavenging activity was measured according to the method of Robak and Gryglewski [7] with some modifications. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 1ml of nitroblue tetrazolium (NBT, 156 µM), 1 ml of reduced nicotinamide adenine dinucleotide (NADH, 468 µM) 3 ml of HEPD solution of different concentrations (50, 100, 200, 400, 800, 1000 1200 µg/ml) were mixed. The reaction was initiated by adding 100 µl of phenazine methosulphate (PMS, 60 µM). The reaction mixture was incubated at 25˚C for 5 min, followed by measurement of absorbance at 560 nm. The percentage inhibition was calculated by using Eq. (1).

3.1.5. *Reductive ability*

Reducing power of the test samples was determined on the basis of the ability of their antioxidant principles to form colored complex with potassium ferricyanide, TCA $FeCl_3$ it was measured by the method reported by Jayprakash *et al.* [8]. 1 ml of different concentrations (25, 50, 100, 200, 400 µg/ml) of the extract fractions were mixed with potassium ferricyanide (2.5 ml, 1%) 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 min. 2.5 ml TCA (10%) was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant was taken out to this 2.5 ml water 0.5 ml $FeCl_3$ (0.1%) were added absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated higher reducing power.

3.2. *Estimation of total phenolic (pyrocatechol gallic acid) compounds*

Total soluble phenolic in the HEPD is determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton [9]. Briefly, 0.1ml of extract in distilled water

(contains 1mg of each extract) was transferred into 100ml Erlenmeyer flask then final volume was adjusted to 46ml by addition of distilled water. Afterwards, 1ml of Folin – Ciocalteu reagent (FCR) was added to this mixture after 3minutes. 3ml of Na_2CO_3 (2%) was added. Subsequently, mixture was shaken on a shaken for 2 hours at room temperature then absorbance was measured at 760nm. All the tests were performed in triplicate the results averaged. The concentration of total phenolic compounds in HEPD was determined as microgram of pyrocatechol equivalent by using Eqs. (2) and (3) that were obtained from standard pyrocatechol graph.

$$\text{Absorbance} = 0.001 \times \text{pyrocatechol } (\mu g) + 0.0033 \quad (2)$$

$$\text{Absorbance} = 0.0053 \times \text{gallic acid } (\mu g) - 0.0059 \quad (3)$$

3.3. *Estimation of total flavonoid content*

The total flavonoid content was determined using the Dowd method [10]. 2mL of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.1 mg/mL). Absorption readings at 415 nm using spectrophotometer were taken after 10 minutes against a blank sample consisting of a 2 ml extract solution with 2 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin (25 - 200 µg/2ml methanol) as the standard. Total flavonoid content is expressed as µg of quercetin equivalents (QE)/mg of extract.

3.4. *Determination of vitamin C*

25.00 ml (containing 25 mg of vitamin C) of vitamin C standard solution to a 125 ml Erlenmeyer flask is taken 10 drops of 1% starch solution is added. Titrate the solution against iodine solution (0.125% iodine, 1% potassium iodide) with 1.00 mg/ml ascorbic acid (made fresh) until the endpoint is reached (first sign of blue color that persists after 20 seconds of swirling the solution).The volume of iodine solution required is noted. Then 25 ml of HEPD extract (containing 25 mg of extracts) is titrated with iodine solution. The volume of iodine solution required is noted. The amount of vitamin C present in different fruit extracts is determined by the comparison of required iodine solution.

3.5. *In vivo antioxidant study*

3.5.1. Preparation of test compound

Stock solution was prepared by dissolving 0.36 gm of the HEPD in 4.8 ml of distilled water. 0.4 ml of the stock solution per 100 gm rat is given orally so that the dose would be 300 mg/kg body weight.

3.5.2. Animals

Male Wistar albino rats (120g ±20) were used for the present study. They were maintained at standard laboratory conditions fed with commercial pellet diet (Hindustan

Lever, Kolkata, India) water *ad libitum*. The experiments were performed based on animal ethics guidelines of Institutional Animals Ethics Committee.

3.5.3. Experimental design

After seven days of acclimatization, the rats were divided into four groups (n=6). Treatment was done for 8 days as follows:

Group I**:** Normal control (0.9% normal saline; 1 ml/kg i.p.)
Group II**:** CCl_4 control (CCl_4: liquid paraffin (1:2); 1ml/kg i.p.)
Group III**:** CCl_4 + HEPD (300 mg/kg/day; p.o).
Group IV**:** CCl_4 + standard drug Silymarin (25 mg/kg/day; p.o).
Group II-IV received CCl_4 in liquid paraffin (1:2) (1.0 ml/kg i.p.) once in every 72 h.

After 24 h of the last dose, blood was collected from retro-orbital plexus under ether anesthesia. The blood samples were allowed to clot and the serum was separated by centrifugation at 2500 g at 37° C and was used for biochemical estimation. All the animals were then sacrificed and liver tissues were collected for the evaluation of *in vivo* antioxidant and other studies.

3.5.4. Estimation of biochemical parameters

Serum was analysed for various biochemical parameters like serum glutamic oxaloacetic (SGOT), serum glutamic pyruvic transaminase (SGPT) activities [11], and alkaline phosphatase [12]. The total protein concentration and total bilirubin were also measured by the method of Lowry *et al.* [13] and Mallay and Evelyn [14], respectively. All the analyses were performed by using commercially available kits from Span Diagnostics Ltd.

3.5.5. Estimation of lipid peroxidation (LPO), enzymic (CAT, SOD) non-enzymic (GSH) antioxidant system

Tissue sample preparation for LPO, GSH SOD assay: 1 g of liver tissue was collected from each experimental rat, washed in normal saline and soaked in filter paper. The tissues were then homogenized in 10 ml of 0.15 M tris buffer (pH-7.4) and centrifuged at 3000 g at 4° C for 30 min. The supernatant was collected was taken for lipid peroxidation, glutathione superoxide dismutase assay.

Tissue sample preparation for catalase assay: 900 mg of liver tissue was collected from each experimental rat, washed in normal saline soaked in filter paper. The tissues were then homogenized in 3.0 ml M/150 phosphate buffer (pH-7.0) and centrifuged at 3000 g at 4° C for 1 hr. The supernatant collected was taken for the assay.

3.5.6. Lipid peroxidation

Lipid peroxidation (LPO) was assayed according to the method of Okhawa *et al*. [15]. To 1ml of tissue homogenate, 1ml of normal saline (0.9% w/v) and 2.0 ml of 10%TCA were added and mixed well. The mixture (3000 g) was then centrifuged at room temperature for 10 min to separate proteins. 2 ml of supernatant was taken, 0.5ml 1.0% TBA was added to it followed by heating at 95° C for 60 min. to generate the pink colored MDA. OD of the samples was measured at 532 nm using Beckman DU 64 spectrophotometer. The levels of lipid peroxides were expressed as nM of MDA/mg wet tissue using extinction co-efficient of 1.56×10^5 M^{-1} cm^{-1}.

3.5.7. Superoxide dismutase (SOD) activity assay

Superoxide dismutase (SOD) activity was assayed according to the method of Marklund and Marklund [16]. The liver homogenates were prepared in Tris (ethylenediamine tetraacetic acid) buffer centrifuged for 40 min at 10000 r.p.m at 4°C, the supernatant was used for the enzyme assay. 2.8 ml Tris-EDTA (composed of 49.78 mM Tris 0.0012 mM EDTA; pH 8.5) 100µl Pyrogallol (2mM) were taken in the cuvette scanned for 3 min at 420 nm wavelength. Then 2.8 ml Tris-EDTA (pH -8.5), 100µl Pyrogallol, 50µl tissue homogenate were taken and scanned for 3 min at the same wavelength. One unit of SOD activity is the amount of the enzyme that inhibits the rate of auto oxidation of pyrogallol by 50% and was expressed as Units/mg protein/min. The enzyme unit can be calculated by using the following equations:

$$\text{Rate}(R) = (\text{final OD} - \text{initial OD})/3 \text{ min} \quad (4)$$

$$\% \text{ of inhibition} = \{(\text{blank OD} - R)/\text{lank OD}\} \times 100 \quad (5)$$

$$\text{Enzyme unit (U)} = (\% \text{ of inhibition}/50) \times \text{common dilution factor.} \quad (6)$$

[50% inhibition = 1 U]

3.5.8. Catalase (CAT)

Catalase activity was measured based on the ability of the enzyme to break down H_2O_2. 10 µl sample was taken in tube containing 3.0ml of H_2O_2 in phosphate buffer (M/15 phosphate buffer; pH-7.0). Time required for 0.05 optical density changes was observed at 240nm against a blank containing the enzyme source in H_2O_2 free phosphate buffer (0.16ml H_2O_2 is 30% w/v was diluted to 100ml of phosphate buffer). The absorbance was noted at 240nm after the addition of enzyme; Δt was noted till OD was 0.45. If Δt was longer than 60 seconds, the procedure was repeated with more concentrated enzyme sample. Reading was taken at every 3 second interval [17]. A unit catalase activity is the amount of enzyme that liberates half the peroxide oxygen from H_2O_2 solution of any concentration in 100 seconds at 25^0C which is determined by CAT activity expression:

Moles of H_2O_2 consumed/min (units/mg)=$2.3/\Delta t$ x ln ($E_{initial}/E_{final}$) x 1.63x 10^{-3} (7)

with E = optical density at 240nm,

Δt = time required for a decrease in the absorbance.

3.5.9. Reduced glutathione (GSH)

Reduced glutathione (GSH) activity was assayed according to the method of Ellman [18]. Reduced Glutathione was estimated spectrophotometrically by determination of DTNB (Dithiobis-(2-nitrobenzoic acid)) reduced by SH-groups, expressed as µg/mg wet tissue.

To 0.1 ml of different tissue samples, 2.4 ml of 0.02 M EDTA solution was added and kept on ice bath for 10 min. Then 2 ml of distilled water and 0.5 ml of 50 %w/v TCA were added. This mixture was kept on ice for 10-15 min, then centrifuged at 3000 g for 15 min. To 1 ml of supernatant, 2.0 ml of Tris buffer (0.4M) was added. Then 0.05 ml of DTNB solution (Ellman's reagent; 0.01M DTNB in methanol) was added and vortexed thoroughly. OD was read (within 2-3 min after the addition of DTNB) at 412 nm in spectrophotometer against a reagent blank. Appropriate standards were run simultaneously.

3.6. Statistical analysis

Linear regression analysis was used to calculate IC_{50} values wherever needed. All the results are shown as average ± S.E.M. Data was statistically evaluated by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's test using instat software. $P< 0.05$ was considered as statistically significant.

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4. Results and Discussion

4.1. In vitro antioxidant activity

4.1.1. *DPPH radical scavenging activity*

The fruit extract demonstrated H-donor activity. The DPPH radical scavenging activity was detected and compared with α-tocopherol. The IC_{50} values for HEPD and α-tocopherol are 160µg/ml 42µg/ml, respectively. DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphenylpicryl hydrazine, with the addition of the extract in a concentration-dependent manner.

4.1.2. *Nitric oxide*

HEPD effectively reduced the generation of nitric oxide from sodium nitroprusside. The extract showed nitric oxide scavenging activity (IC_{50} 1.4mg/ml) that of standard curcumin was 45 µg /ml. Scavenging of nitric oxide radical is based on the generation of nitric oxide

from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent.The absorbance of the chromophore is measured at 546 nm in the presence of the fractions. HEPD decreases the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*.

4.1.3. *Hydroxyl radical scavenging activity*

The scavenging activity of HEPD (IC_{50} 1.05mg/ml) was lower than that of curcumin (5µg/ml). Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of deoxyribose by the free radicals generated by the Fenton reaction. The oxygen derived hydroxyl radicals along with the added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid.

4.1.4. *Superoxide radical scavenging activity*

The extract shows dose dependent free radical scavenging activity. The percentage inhibition is shown in Fig. 1. The scavenging activity of HEPD (IC_{50} 1.115mg/ml) was lower than that of standard curcumin (5µg/ml). Superoxides are produced from molecular oxygen due to oxidative enzyme of body as well as via non-enzymatic reaction such as autoxidation by catecholamines. In the present study, superoxide radical reduces NBT to a blue colored formazan that is measured at 560 nm.

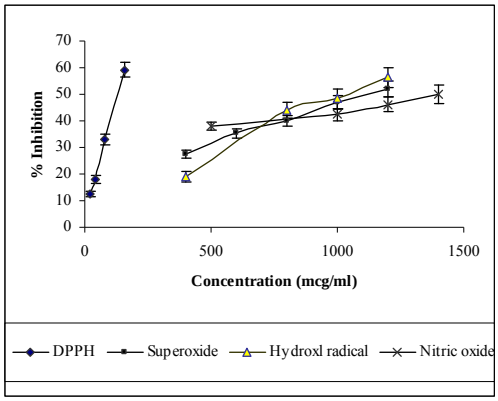

Fig. 1. *In vitro* antioxidant activity of HEPD fruit in different models.

4.1.5. *Reductive ability*

Fig. 2 shows the reductive capabilities of HEPD when compared to the standard, BHT. Like the antioxidant activity, the reducing power increased with increasing amount of the extract. For the measurement of the reductive ability, the Fe^{3+}-Fe^{2+} transformation was

investigated in presence of the extract. Presence of reductants causes the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} form. This Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm.

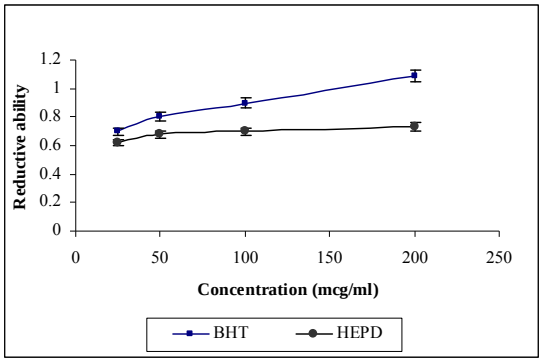

Fig. 2. Reductive ability of HEPD fruits.

4.2. *Estimation of total phenolic (pyrocatechol gallic acid) compounds*

The total phenolic compound of HEPD is shown in Fig. 3**.** Total pyrocatechol and gallic acid compounds of HEPD are 6.2 and 2.906 µg/mg, respectively. Total phenolic assay by using Folin-Ciocalteu reagent is a simple, convenient reproducible method. It is employed routinely in studying phenolic antioxidants.

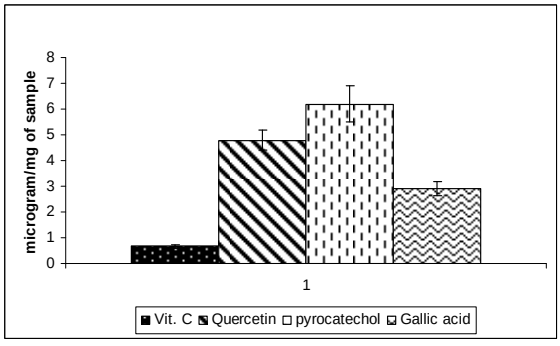

Fig. 3. Total phenolic, flavonoid vitamin C content of HEPD fruit.

4.3. *Estimation of total flavonoid content*

Flavonoids are large class of benzo-pyrone derivatives, ubiquitous in plants exhibit antioxidant activity. The flavonoid content of different fruits extract is shown in Fig. 3. Total flavonoid content of HEPD is 4.79 µg of quercetin equivalents (QE)/mg of extract.

The antiradical property of flavonoids is directed mostly toward hydroxyl, superoxide as well as peroxyl and alkoxyl radicals. Furthermore, as these compounds present a strong affinity for iron ions (which are known to catalyze many processes leading to the appearance of free radicals), their antiperoxidative activity could also be ascribed to a concomitant capability of chelating iron.

4.4. *Determination of vitamin C*

Vitamin C content of HEPD is 0.66mg/g (Fig. 3). As a water-soluble antioxidant (can not be stored be our body), vitamin C is in a unique position to "scavenge" aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids.

4.5. *In vivo antioxidant study*

4.5.1. *Estimation of biochemical parameters*

Biochemical parameters (SGOT, SGPT, ALP, total protein, total bilirubin) are shown in Table 1. The level of SGPT, SGOT, ALP, total protein, total bilirubin is restored towards the normal value in HEPD treated carbon tetrachloride intoxicated rats. Peroxidative degradation of cellular membrane due to CCl_4 induction causes functional morphological changes in it resulting in cellular leakage loss of functional integrity of the membrane. It was found from Table 1 that in CCl_4 control group by the substantial increase in the level of serum marker enzymes (SGOT, SGPT and ALP). The reduction of the level of total proteins in CCl_4 challenged animals (Table 1) is attributed to the damage produced localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis. The rise in protein levels in the treated groups suggests the stabilization of endoplasmic reticulum leading to protein synthesis.

4.5.2. *Estimation of lipid peroxidation (LPO), enzymic (CAT, SOD) non-enzymic (GSH) antioxidant system*

Reduced activities of enzymic (CAT, SOD), non-enzymic (GSH) antioxidant system lipid peroxidation (LPO) level of liver homogenate were summarized in Table 2. The level of CAT, and SOD reduced glutathione is increased LPO decreased towards the normal value in HEPD treated carbon tetrachloride intoxicated rats. The antioxidant activity or the inhibition of the generation of free radical is important in the protection against CCl_4-induced liver lesion. The level of lipid peroxide is a measure of membrane damage alterations in its structure function. The level of MDA, which is one of the end products of lipid peroxidation in liver tissue, was found to be high in CCl_4 control group (shown in Table 2) implying enhanced lipid peroxidation leading to tissue damage failure of antioxidant defense mechanisms against free radicals. Treatment with HEPD Silymarin

significantly reversed these changes. Our body has an effective defense system against free radical induced damage. It consists of a set of endogenous antioxidant enzymes, two of the key components of which is catalase (CAT) Superoxide dismutase (SOD). Regarding non-enzymic antioxidants, reduced glutathione (GSH) is a critical determinant of tissue susceptibility to oxidative damage. It is an intracellular reductant which is extensively found in cells. It protects cells against electrophilic attacks by xenobiotics such as free radicals peroxides. In the present study it is observed that CCl_4 deplets GSH concentration in the rat livers. HEPD Silymarin treatment reverses this effect, which may be due to *de novo* GSH synthesis or GSH regeneration.

Table 1. Effect of hydromethanolic extract of *P. dactylifera* (HEPD) on serum enzyme levels, total bilirubin, total protein of CCl_4 intoxicated rats.

Treated Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/100 ml)	Total protein (mg/dL)
Carbon tetrachloride	138.87±10.12$^{\#}$	125±9.44$^{\#}$	44.66±4.02$^{\#}$	3.12±0.2$^{\#}$	4.21±0.38$^{\#}$
Normal control group	54.299±1.26	24±1.84	9.51±1.12	1.10±0.19	7.16±0.708
Silymarin (standard)	58.65±1.66*	28±1.98*	12.23±1.19*	1.15±0.16*	6.49±0.649*
Date palm	123.08±1.518*	82±6.33*	29.09±2.98*	3.05±0.1	4.47±0.447*

Values are mean ± S.E.M.; *n*=6 in each group. Drug treatment was done for 8 days.
$^{\#}P$<0.001 CCl_4 treated group compared with normal group; $^{*}P$<0.05 Experimental groups compared with control group; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

Table 2. Effect of hydromethanolic extract of *P. dactylifera* (HEPD) on LPO, GSH and CAT levels of CCl_4 intoxicated rats.

Treated Group	Lipid Peroxidation [MDA content (in nano mole/milligram)]	GSH content (μg/mg wet tissue)	CAT (μM of H_2O_2 decomposed/min/mg wet tissue)	SOD (U/mg wet tissue)
Carbon tetrachloride	99.31±5.59$^{\#}$	2.06±0.08$^{\#}$	0.478±0.0250$^{\#}$	6.4±0.62$^{\#}$
Normal	20.78 ± 0.757	5.21±0.29	1.045±0.0434	10.6±0.9.6
Silymarin (standard)	39.40±4.0398*	5.1±0.22*	0.966±0.013*	10.3±0.92*
Date palm	75.28±7.35*	2.53±0.29*	0.55±0.007*	6.8±0.31

Values are Mean ± S.E.M.; n=6 in each group. Drug treatment was done for 8 days.
$^{\#}P$<0.001 CCl_4 treated group compared with normal group; $^{*}P$<0.05 Experimental groups compared with control group; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett's test.

5. Conclusion

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, alzheimer, hepatic damage etc.

Antioxidants fight free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. HEPD decreases the amount of nitrite generated from the decomposition of sodium nitroprusside, scavenged the hydroxyl radicals prevented the degradation of deoxyribose, the reducing power of HEPD extract, like its antioxidant activity, increases with increasing concentration which implies that extract is capable of donating H atoms in a dose dependent manner. Phenolics are ubiquitous secondary metabolites in plants and possess a wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical scavenging activities. These also decrease cardiovascular complications. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. HEPD showed significantly higher inhibition percentage (stronger hydrogen-donating ability) positively correlated with total phenolic content.

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, and anticancer activities. Vitamin C is an excellent source of electrons; therefore, it can donate electrons to free radicals such as hydroxyl and superoxide radicals to quench their reactivity

In most of the developing countries, the incidence of viral hepatitis is more. So, the investigation for an efficient hepatoprotective drug from the natural resource is an urgent necessity. The changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis. CCl_4 is therefore a useful tool for the induction of hepatic damage in experimental animals. The ability of hepatoprotectivity of HEPD to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its protective effects.

Hence, the present investigation suggests that HEPD shows good antioxidant activity, reducing power, free radical scavenging activity and hepatic protection. Phytochemical screening of the crude HEPD reveals the presence of flavonoids, saponins, tannins steroids. Thus these *in vitro* in *vivo* antioxidant potential of HEPD may be due to the presence of these phytoconstituents and vitamin C.

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