Original article:

Nutritional and toxicological analysis of *phoenix dactylifera* (date palm) powder used as a drink

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Abstract:

Objective*:** *Phoenix dactylifera* fruit widely used in Muslim countries**. They have** religious attachment to it and also it had many health benefits. It is essential to investigate nutritional values of *Phoenix dactylifera* fruit in dried powder form and to find out any toxicological changes during processing of this dried powder drink. ***Method*:** Dried date powder was prepared from fresh fruit by drying it and its nutritional value and toxicological studies were carried out. The acute oral toxicity study of date powder was conducted on wistar strain rats by oral route in a dose of 0.5g/kg body weight and 0.1g/kg body weight. ***Result: The results of biochemical analysis of dehydrated date powder exhibited that it possesses high energy value of 311 Kcal, carbohydrate 70.5%, dietary fiber 7.3%, fat content 2.1%, protein 2.6%, and mineral content 2.2%. Whereas, total sugar was found to be as 63.2% and moisture content 2.3%. The test drink showed no sign of toxicity or death during the whole observation period (fourteen days) in rats. No group of animal showed any unusual change in behavior or in locomotors activity. The macroscopic studies of vital organs i.e. heart, liver, spleen, lungs and kidneys exhibited that they were normal. ***Conclusion:*** As a result of these studies it can be concluded that from processing of fresh fruit to dried powder along with its nutritional and toxicological studies no hazardous material like toxins were produced. So it can be used safely.

Keyword: *Phoneix dactylifera* fruit; acute oral toxicity studies; nutritional values.

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Introduction

It is well reported that a human body need a variety of essential vitamins and minerals to carry out important roles to keep it well. Now a day's mostly people for their good health, higher energy, more strength and fitness, like to add health drinks in their diet to improve their daily nutrition intake. In market a variety of nutritional drinks are available which contain high calories and it is reported that calories improve the quality of health and to lengthen lifespan[1]. People who are looking to lose weight should avoid health drinks and look for more natural foods like fruits and vegetables which are packed with vitamins and minerals and are low in calories as compared to any other food. In the light of this background the new and natural compounds is the focus of current research.

Phoenix dactylifera (date palm) belongs to a family *Arecacean* is the oldest edible sweet desert fruit found in countries around the Arabian Gulf[2]. Its fruit

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contain numerous medicinal properties while non fruit material is also of great value and used for many other purposes[3]. The phytochemical constituents of *P. dactylifera* include alkaloids, flavonoids, steroids, tannins, estertepens, carbohydrates, phenolic acids and at least six vitamins including a small amount of vitamin C, and vitamin B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A[4]. Enzymes such as phytase, invertase and peroxidase have been isolated in dates[5]. *P. dactylifera* also posses numerous medicinal properties like for treatment of anaemia, stroke, antiulcer activity, anticancer activity, anti-diarrhoeal, hepatoprotective activity, antimutagenic activity, antioxidant activity, anti-inflammatory activity, antiviral activity and antihyperlipidemic activity[6]. The various parts of this plant are widely used in traditional medicines for the treatment of various disorders which include memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders[5]. Despite the numerous health benefits of *P. dactylifera* there is no market available energy drink based on it. Keeping in view the above facts we decided to evaluate it scientifically as nutritional supplement along with its toxicity studies.

Experimental

Chemicals

Table Sugar (Madina Sugar Mills (PVT) Limited, Pakistan), Sodium Chloride (Riedel-de Haën Chemicals Germany), CMC (Sigma Chemicals US), $CuSO_4$ (Allied Signals Chemicals Europe), K_2SO_4 (Riedel-de Haën Chemicals Germany), H_2SO_4 (Merck Private Ltd. Germany), NaOH (Merck Private Ltd. Germany), Pet-Ether (M-Tedia Chemicals US), Diethyl Ether (M-Tedia Chemicals US).

Instruments

Dryer (PCSIR Labs Complex Pakistan), Grinder (Turchmir Engineering company Pakistan), Fluidized Bed Dryer (Technology International Pakistan), Ribbon Type Blender (Alwajid Enterprises Pakistan), Soxhelet Model #: SE-1/02 (PCSIR Labs Complex Pakistan), Oven Model #: 101-1 (PCSIR Labs Complex Pakistan).

Preparation of sample

P. dactylifera (100kg) fruit was purchased from local market and identified by botanist Nida saleem Dept. of Food Technology, PCSIR Labs Complex Karachi, Pakistan (specimen number: PD-094-2012). The dust particles and adhered material from fruit were removed with water in automatic fruit washing machine. The cleaned *P. dactylifera* fruit were transferred to Steam Jacketed Reactor for boiling with water at 90-95 ^{0}C for 45-50 minutes. The boiled dates were then placed on trays in a force circulating hot air cabinet dryer, where they were dehydrated at 55 ^{0}C for a period of 16hr. After dehydration, the seeds were removed from partially dehydrated dates and chopped through a continuous stain less steel chopper. Sodium Benzoate is added and thoroughly mixed for 10 -15 minutes The chopped dates were again placed in trays of force circulating hot air cabinet dryer, where they were again dehydrated at 55 ^{0}C for a period of 12 hrs. After the completion of dehydration process, the chopped dates were converted into solid mass having a brown color. At this stage 15-16 Kg dried solid mass was ground in a Pin Grinder to obtain 100-120 mesh size powder, this powder was then further dried in a Fluidized Bed Dryer at 50^{o}C for 30-35 minutes where 4-5 % moisture contents were removed. Total 15 kg of dry powder is obtained from 100 Kg of *P. dactylifera* Fruits. The Ribbon Type Blender mixer was used for thorough mixing of dried powder with 8-10 kg of sugar 0.5-1 kg of Sodium chloride and 250-300g of Carboxy Methyl Cellulose. The fine grinder was used for further grinding up to 220-250 mesh size of formulated *P. dactylifera*. The finished *P. dactylifera* powder was packed in a 25-30g of food grade sachet packing, which is sufficient for 120 ml/of date drink.

Nutritional Value Assessment

The dehydrated fine grounded date powder was processed according to the standard method of analysis. The method used was consistent in both standardization and determination[21].

Moisture

Accurately weighed 2.5-5g of sample was taken into a pre-dried nickel or stainless steel dish. Samples were spread as thinly as possible over the base of the dishes to provide the maximum drying area for the removal of moisture content. The dishes along with contents were put in an oven maintained at 70 ± 2°C and dried for 04 to 05 hours. After the completion of drying process, the samples were cooled in a dessicater and weighed. After weighing once, dishes were re-dried for further thirty minutes, removed, cooled and weighed. Continued drying until a constant weight had been reached. The moisture content was calculated from the weight loss of the samples [21,22].

Protein

Protein was determined by adapting the standard method. 0.7-2.0g sample was digested with 0.5g of Copper sulfate and 5g of potassium sulfate respectively, with addition of 25ml of sulfuric

acid. The digestion flask was heated gently until the frothing ceased and briskly boiled cleared solution was obtained. After cooling, 200ml of distilled water and sodium hydroxide were added. Immediately the flask was connected to a distilling bulb on a condenser, with a tip of condenser immersed in acidic solution and 5-7 drops methylene red indicator in a receiver. To mix the contents thoroughly, the flask was rotated and then heated until NH_3 had been distilled ($\geq$ 150ml distillate). The receiver was then removed, washed up of the condenser, and the titrated excess standard acid is distillate with the standard NaOH solution was corrected for blank determination on reagents[21].

Fat

2.5-5g of sample was weighed directly on a filter extraction thimble, and the end of thimble was plugged with fat-free cotton wool, then it was placed in a central siphon portion of a Soxhelet. 40ml of pet-ether and diethyl-ether were taken in a flask and connected to Soxhelet siphon and condenser, refluxed for 5hours and distilled of mixed ether and the flask was placed in an oven for distillation at 100-105˚C. The flask was then cooled and weighed after 3hours of drying period. Fat was calculated from the weight of material in a receiver flask[23].

Fiber

225ml of ethanol (90%) was added to digested test portion of fruit sample at 60-65˚C. After removal from the water bath, the beaker was left for precipitation for at least one hour at room temperature with a covering of aluminum foil to prevent from evaporation losses. The precipitated sample was then shifted to post dried crucibles and was dried overnight in an oven at 100-105˚C. After cooling the crucibles in a dessicater, crucibles were weighed containing the fiber residue and the celite nearest to 0.1mg (celite bed had been formed in previously tarred crucibles using 15ml of 78% ethanol). The weight of residue was calculated by subtracting the weight of dried crucibles with celite[21].

Reducing Sugar and Sucrose content

Reducing sugar in date powder was determined by AOAC[21] method of reducing sugars in honey whereas the total sugar was determined by the anthrone method [24]. A 10% of homogenized solution of samples was prepared. First, 25ml of mixed Soxhelet solutions [12.5 ml of Fehling A (copper sulfate solution) + 12.5 ml of Fehling B (alkaline tartrate solution)] were taken into 300-400 ml conical flask (method used was consistent in standardization and determination). Both the reagents i.e., Fehling A and B were pre-standardized with 1% Standard invert sugar solution. 10% homogenized solutions of all the samples were titrated against mixed Soxhelet solutions by placing 25 ml of Soxhelet solutions on a hot plate with continuous heating and stirring commenced to titrate adding 0.5 ml quantities every two seconds without went-off the boiling. A distinct reddening occurred near the end-point. At this stage the addition of a titer reduced to the rate of 0.1 ml. The end-point was taken as the appearance of the bright red color of copper-oxide in the solution. Titration was repeated by adding whole of the initial test titer less 0.5 cm^3. The end-point was arranged so as it falls within a three to four minutes of boiling period. For inversion, 40 ml of 10% clarified sample solutions were taken in 50 ml conical flask and 05 ml of concentrated Hydrochloric acid was added and the content was incubated at 55-60˚C for exactly 10 minutes. After incubation, the sample contents were cooled and tittered same as mentioned above the process before inversion. Sucrose content was determined after inversion by multiplying 0.95 (S-R). Whereas 0.95 is the factor, S is the reducing sugar content of sample after inversion and R is the reducing sugar content of sample after inversion/ Total sugar content of a sample[21].

Mineral content

Accurately weighed 2.5-5g of date powder was taken in a pre-dried and weighed crucible. The sample was charred over a high Bunsen flame until whole sample turned in black with no fume ignition and then it was ashed at 500-550˚C until a grey ash was formed. After 5-6 hours, the sample was removed and allowed to cool in a dessicater. Ashed for further few minutes and reweighed after cooling. Mineral content was determined by adapting the standard method[21,22].

Carbohydrate

Accurately weighed 0.1g of sample was hydrolyzed for three hrs with 5 mL of 2.5 N HCl and cooled to room temperature. Neutralization was done with solid sodium carbonate until the effervescence ceased and volume made up to 100 mL and centrifuged. After centrifugation the supernatant was collected and 0.5 to 1 ml aliquots were taken for analysis. Standard was prepared by taking dilutions 0-1 ml of the working standard whereas '0' served as blank and the volume was make up to 1 ml by adding distilled water. Anthrone reagent was added to dilutions

and left for heating in a boiling water bath. Cooled rapidly and the green colored formed was read at 630 nm against blank. Standard graph was plotted verses concentration and absorbance and total carbohydrate present in a sample was calculated from the graph[25].

Energy Value

The energy calculation of date powder was determined by Atwater method by using factors to calculate energy from protein, fat, and carbohydrate[26].

Toxicity studies

Animal selection

The acute oral toxicity was conducted on wistar strain rats of either sex (150-210g) acquired from the Animal House, PCSIR Labs complex Karachi. The experimental procedures relating to the animals were approved by ethical committee of PCSIR before starting the study. The animals used in the experiment were selected at random and marked on the tails for individual identification. All of the cages were located in a room at temperature approximately 23 °C with constant humidity. The room is regulated with cycles of 12 hours of light and 12 hours of darkness. The animals were acclimated to the laboratory environment for a week earlier before starting the experiment. Drinking water and food were provided *ad labium* through the experiment, except for the short fasting period where the drinking water was still in free access but no food supply was provided 12 hours prior to treatment.

Acute oral toxicity test

The acute oral toxicity of *P. dactylifera* was evaluated in rats according to Organization for Economic Co-operation and Development (OECD) guidelines[27]. Animals were divided into three groups (n=6) Group I and II were treated as test groups and received *P. dactylifera* at 0.5g/kg and 0.1g/kg body weight. Group III received distilled water only and served as control group. All drugs were administered through the oral route following the overnight fasting period. Food was provided to all animals approximately 2-3 hour after treatment. The animals were observed strictly for any indications of toxicity effect during the first 30 minutes followed by first six hours and daily further for a period of 14 days. Visual observations were focused on any mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness, impairment in food and water consumption, postural abnormalities and hair loss[21]. This study was obtained local ethical approval from ethics committee.

Result

The results of biochemical analysis of dehydrated date powder having high energy value of 311 Kcal, carbohydrate 70.5%, dietary fiber 7.3%, fat content 2.1%, protein 2.6%, and mineral content 2.2%. Whereas, total sugar was found to be as 63.2% and moisture content 2.3% (Table: 1).

Table 1: Nutritional Value of Dried Powder of *Phoenix dactylifera* Fruit

S. No.	Parameters analysed/100g	*Phoenix dactylifera*
	Energy (Kcal)	311
	Carbohydrates	70.5 g
	Sugars	63.2 g
	Reducing Sugar	12.1 g
	Sucrose	51.0 g
	Dietary Fiber	7.3 g
	Protein	2.6 g
	Fat	2.1 g
	Mineral	2.2 g
	Moisture	2.3 g

The acute oral toxicity test of dried date powder in a dose of 0.5g/kg body weight and 0.1g/kg body weight p.o. showing no sign of toxicity during the whole observation period (fourteen days) and showed no signs of distress or toxicity or death. No group of animal showed any unusual change in behavior or in locomotors activity (Table: 2, 3).

Table 2: Acute Oral Toxicity Test of Dried Powder of *Phoenix dactylifera*

Sr. #	Groups	No. of animals	Average weight	No. of animals died	No. of animals survived	% age	
						Mortality	Survival
1	I	6	166.00g	Nil	6	0	100
2	II	6	180.50g	Nil	6	0	100
3	III	6	207.00g	Nil	6	0	100

Table 3: Post Feeding Physical and Behavioral Changes in Test and Control Groups

Sr. #	General Signs	Test group I (0.5mg/kg)		Test group II (0.1mg/kg)		Control group	
		6hr	14hr	6hr	14hr	6hr	14hr
	Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal
	Eyes	Normal	Normal	Normal	Normal	Normal	Normal
	Behavioral patterns	Normal	Normal	Normal	Normal	Normal	Normal
	Salivation	Normal	Normal	Normal	Normal	Normal	Normal
	Sleep	Normal	Normal	Normal	Normal	Normal	Normal
	Diarrhea	Normal	Normal	Normal	Normal	Normal	Normal
	Coma	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed
	Tremors	Not observed	Not observed	Not observed	Not observed	Not observed	Not observed

The macroscopic studies of vital organs i.e. heart, liver, spleen, lungs and kidneys exhibited that they were normal. As a result of these studies it can be concluded that from processing of fresh fruit to dried powder along with its nutritional and toxicological studies no hazardous material like toxins were produced. So it can be used safely.

Discussion

In this competitive world everyone do heavy work mentally as well as physically for successive and happy life so they need more energy as fuel for body. Although body made several nutrients itself but to get essential nutrients it has to relay on some other sources. The safe and cost effective source of nutrients for humans is the most edible part of tree that is fruit which provide energy to body for its good health, growth, development and wellbeing[7]. Now a day peoples are conscious for their health and more interested towards herbal products. WHO reported that 80% world population relay on herbs as they are low cost, safe with least side effects[8, 9]. In the light of these facts a drink was formulated from *P. dactylifera* fruit to utilize it in our daily diet to provide more essential nutrients to body as it can perform its daily activities without getting tired or stressed. The reason for selection of this plant is that it is indigenous to Pakistan in excellent export quality and Muslims had religious attachment to this fruit as it being used to break the fast during Ramadan. The nutritional analysis and acute oral toxicity of formulated drink from was carried out. The results of nutritional analysis indicate that the formulated date powder having high energy value of 311 Kcal, carbohydrate 70.5%, dietary fibre 7.3%, fat content 2.1%, protein 2.6%, and mineral content 2.2%. Whereas, total sugar was found to be as 63.2% and moisture content 2.3%. Commonly the major portion of the health drink is carbohydrates (for energy), protein (for muscle building), fat (for basic functions), vitamins and minerals (for cell functions). Our test drink possesses all these nutrients that replenish energy and revitalize the body instantly. Numerous reports on chemical analysis of *P. dactylifera* reported that it possess high nutritional value up to 70 % sugar, more than 80% of magnesium, 70% of sulfur, 25% of potassium, 20% of calcium, high percentage of carbohydrate, fat which comprising 14 types of fatty acids, 15 salts and minerals, protein with 23 different amino acids, six vitamins and a high percentage of dietary fiber, iron, manganese, copper, zinc, magnesium, cobalt, copper, fluorine, potassium, phosphorous and sodium[10-11]. Another study conducted on nutritional value of *P. dactylifera* karnel reported that *P. dactylifera* karnel possesses similar constituents that are present in *P. dactylifera* fruit this study also supports our results[12]. It is also reported that 1 to 2% calorie of energy obtain from diet in fat form is enough for body as excess fat may cause cardiac problems[13]. While some other studies reported that the fibers had health-promoting properties as it lowering the serum cholesterol, risk of coronary heart disease, blood pressure, constipation, diabetes, breast cancer and detoxification of poisonous metals[14-17]. All these studies supports our biochemical results (Table: 1). Chemical properties of a fruit or a product are considered as of great importance in grading, preservation and storage point of view. The higher moisture content facilitates the spoilage and very low moisture content leads to dry fruit which will not be acceptable for consumers. However the decline in moisture content and increase

in sugar content renders the product extremely resistant to fungal spoilage[18]. It is important to note that the ratio of moisture content is related to sugar content, since dates with low moisture content will contain high sugar and vice versa[19]. It can be easily be processed under unfavorable conditions like humus atmosphere, rain fall etc. without any fear of spoilage with improved export potential. The process is safe for protection from dirt, dust, and infestation etc.

Any edible product that comes in market first need safe results from animal studies before human consumption. The acute oral toxicity study is necessary not only for dose range but also for identification of any clinical signs produce by substance that is under investigation. For acute oral toxicity studies oral route was selected in animals for drug administration because it is painless to the animals having less cost the most convenient and normally used method. After giving drugs to all respective groups of animals, they were monitored daily up to fourteen days for any toxic signs and mortality. The results exhibited that *P. dactylifera* is health protecting agent, during the whole observation period rats showed no signs of distress or toxicity or death. No group of animal showed any unusual change in behavior or in locomotors activity. All animals were found actively moving, climbing, jumping over the cage cover and showed no changes in behavior (Table: 2,3). It is reported that food and water consumption is important during the study of safety of a product[20]. It was also observed that food and water intake was similar to all groups indicating that feed and water consumption and its utilization was not disturbed after the administration of *P. dactylifera* formulated drink. Thus, it indicates that there was no disturbance in physiological functions of animals like carbohydrate, protein or fat metabolism. Autopsy was carried out to confirm the safe results and for this purpose heart, liver, kidney, spleen and lungs were macroscopically observed in both control and treated groups. Autopsy findings showed no gross changes. All vital organs i.e. heart, liver, spleen, lungs and kidneys were found normal as a result of which it can be concluded that during processing of fresh fruit in dried powder no hazardous material like toxins were produced.

Conclusion:

The preliminary study about the toxicological and nutritional evaluation of *phoenix dactylifera* (date palm) powder indicates that date powder as a drink is a rich source of nutrients and vitamins and after toxicity study in animals, no toxic results were obtained. After autopsy all vital organs were found normal.

Conflic of interest: None

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