IJFANS
International Journal of Food And Nutritional Sciences

Volume 4, Issue 4, Jul-Sep 2015, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.4, Iss.4, Jul-sep, 2015
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Research Paper *Open Access*

SAFETY AND PROTECTIVE EFFECT OF DATE (*PHOENIX DACTYLIFERA*) SEED EXTRACT AGAINST OXIDATIVE DAMAGE IN RAT

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Received on: 26th August, 2015 **Accepted on: 2nd October, 2015**

ABSTRACT

Oxidative stress results from disequilibrium between the spontaneous productions of reactive oxygen/nitrogen species and the natural antioxidant defense system. It has been related to the development of chronic diseases, neuro-degenerative disorders and aging process. Nowadays, the body antioxidant defense system is constantly challenged by a variety of new environmental pollutants and chemicals, which could overwhelm the antioxidant defense. Because of their antioxidant properties, polyphenols have been suggested as potential candidates to help in maintaining the oxidative balance. Date seed extract (DSE) is among the richest plant sources of antioxidant components, but *in vivo* data on its safety and efficacy are lacking, especially in different organs. Our purpose was to determine the effect of DSE on antioxidant status, oxidative damage and tissue function in male Wistar rats, which were fed a basal diet alone or in which 240mg or 480mg of DSE/kg diet were incorporated, for 13 weeks. All diets were isonitrogenous and isocaloric. Growth rate was higher with DSE diet. Protein, albumin, total cholesterol and HDL cholesterol were slightly decreased with the highest dose of DSE. Creatinine, lactate dehydrogenase, creatine kinase, aspartate aminotransferase, alanine aminotransferase and gamma-glutamyl transpeptidase were decreased in rat serum with DSE. In addition, DSE significantly increased Vitamin E in the muscle, GSH in liver, muscle and heart and reduced damage products of lipid and protein oxidation in liver, muscle and brain. Histopathology did not reveal any abnormalities. An *in vivo* protective effect against oxidative damage of DSE is suggested, possibly related to its antioxidants compounds

Keywords: Date seeds, animal, oxidative status, toxicity, tissue damages

INTRODUCTION

Spontaneously, as a result of cellular activity, reactive oxygen/nitrogen species such as superoxide radicals, hydrogen peroxide, hydroxyl radicals, singlet oxygen, lipid hydroperoxides, peroxynitrite and related species are generated in the body. These reactive species can cause oxidative damages to essential cellular constituents such as membrane lipids, proteins and DNA, which may ultimately result in cell death. Nonetheless, in the normal condition of functioning of the aerobic organisms, endogenous antioxidants like glutathione and exogenous antioxidants taken in through the diet like vitamin E and vitamin C protect them to maintain the oxidative balance. Any factor promoting oxidative disequilibrium can lead to a condition named oxidative stress, which has been identified as one of the major underlying mechanisms for chronic diseases (type 2 diabetes, cancer, cardiovascular diseases, and neuro-degenerative disorders) and the aging process (Saltman, 1989; Sies, 1997; Uttara *et al.*, 2009; Mangge *et al.*, 2014).

Nowadays, compared to the ancient life, it is noteworthy that a variety of new pro-oxidant compounds coming from environmental pollutants and radiations is constantly challenging the antioxidant defense system. This represents a real threat for the balance of the whole body oxidative system.

In order to counteract these deleterious effects, natural antioxidants, among which are polyphenols, were suggested as potential candidates. The interest in polyphenols is largely related to their protection against oxidative stress in humans (Sies, 1997). Polyphenols possess the abilities to interfere with the formation and propagation of free radicals and protect cells and tissues against oxidative damages.

Interestingly, date seeds have been shown to have an excellent nutritional quality (Habib & Ibrahim 2009)

and antioxidant properties due to their high content of phenolics (24.6 g kg^{-1} gallic acid equivalent) and total flavonoids (63.67 g kg^{-1} rutin equivalent) (Habib and Ibrahim, 2011; Habib et al., 2013). Recently, the investigation of the polyphenolic profile of date seed extract revealed that the main compounds were the polyphenolic compounds, which represented up to 5% of the extract (Habib et al., 2014).

Dates are largely cultivated in the Middle East and are one of the most popular staple foods in this region of the world. Date seed is one of the byproducts generated in a huge amount in the production process of dates, and they are being wasted in large quantities or used mainly for animal (camels, cattle, sheep and poultry) feed. Date seeds are also used on a very limited scale in making a caffeine-free beverage with a coffee-like flavor (Rahman et al., 2007). Recently, it has been shown that date seed powder supplemented diet (7 and 14%) was safe in rat after 30 days of treatment and even exerted a protective effect against oxidative damage (Habib and Ibrahim, 2011).

Thus, developing additional applications for date seeds will increase the income of date producers and consequently benefit date-producing countries. The great nutritional properties and health benefits of date seed might give a wide range of potential applications including use as ingredients in the production of some functional food products as well as nutraceuticals.

In this purpose, date seed extract, due to its form, which may facilitate the incorporation in many different matrices and because it concentrates bioactive compounds initially found in date seed, might be the preferable form to expect great impacts on human health.

However, so far, the safety and efficacy of date seed extract have not been studied *in vivo*. Therefore, the objectives of this study were to investigate the safety and antioxidant activity of date seed extract in rats.

MATERIALS AND METHODS

DATE SEEDS

SAMPLING

Date seeds from the Khalas variety were obtained from Al Ain Dates Factory (Al Ain, UAE). The season (summer) of collecting tamr (fully ripe dates) is usually spread over a period of 2–3 months. Samples were collected randomly from tamr batches at the end of the season, with no preference to size, color, appearance or firmness. The seeds were first soaked in water, washed to get rid of any adhering date flesh, air-dried, and ground into coarse powder using a hammer mill.

EXTRACTION

Extraction was performed with a protocol adapted from that described by Mane et al. (Mane et al., 2007).
The extraction of antioxidant compounds from the seed was carried out using methanol/H_2O 50:50, v/v. Date seed powder was mixed with the extraction solvent 1:10 w/v and checked overnight. The supernatant was filtrated using filter paper, then lyophilized until dryness. The lyophilized powder was kept at -80 ^oC until use.

ANIMALS

The protocol used in this study was reviewed by the Animal Research Ethics Committee, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain and was conducted according to the Declaration of Helsinki principles. It was recorded as protocol number A19-12 and was approved on June, the 4^{th}, 2012. The study was conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Institute of health, 1985).

Normal Wistar male rats (43-113 g) were obtained from the College of Medicine and Health Sciences Animal Facility, UAE University, Al Ain.

The rats were housed in plastic cages under controlled conditions of 12-h light/12-h dark cycle, 50% humidity and 25±3ºC.

EXPERIMENTAL DESIGN

The animals were randomly divided into 3 groups. They were fed for 13 weeks before sacrifice. An isocaloric and isonitrogenous basal diet, similar to the American Institute of Nutrition AIN-93G purified rodent diet (Reeves et al., 1993) was used. Two DSE containing diets were prepared by using two different DSE doses: 240mg of DSE/kg diet (DSE1 Diet) and 480mg of DSE/kg diet (DSE2 Diet). During the experimental period, the control group (5 rats) received the basal diet, DSE1 and DSE2 groups (9 rats each) received DSE1 and DSE2 diets, respectively. Water and feed were provided *ad libitum* to the rats.

Since, no human clinical trial using date seeds extract has been conducted so far, other phenolic compounds rich plant extracts have been considered as reference. Grape seeds have been shown to be a very high source of phenolic compounds and grape seed extract has already been more extensively tested in humans. In the recent published meta-analysis (Feringa et al., 2011), the highest dose of grape seed extract which was tested in human was 2000mg/d. No adverse effect was reported, and impact on different cardiovascular risk markers like blood pressure and heart function were described. This dose is equivalent to about 6 mg/d/rat (considering an average weight of 60 kg for humans and an average weight of 0.2 kg for rats), which translates to 240 mg/kg diet of DSE. According to results from the determination of phenolics profile of date seeds (Habib et al., 2014), bioactive compounds represent 5% of the extract. Considering a daily food intake of 25g of diet/day/rat, as it has been described in a previous animal study done by co-authors (Habib and Ibrahim, 2011), the dose of 240mg/kg diet of DSE would provide 0.3 mg/day of phenolic compounds to the animal. By choosing the daily amount of 240 mg/kg diet and 480 mg/kg diet date seeds extract, it can reasonably be considered that potential adverse effects of a high consumption of date seed extract will be detected, if any.

LABORATORY MEASUREMENTS

PREPARATION OF SERUM, PLASMA, TISSUE FRAGMENTS AND HOMOGENATE

At the end of the experimental period, rats were anaesthetized with pentobarbital and killed following blood withdrawal via heart puncture. Blood samples were drawn into dry (for obtaining serum) and heparinized (for obtaining whole blood) tubes. Portions of serum were immediately separated after centrifuging the blood sample and used for measuring the levels of glucose, total cholesterol and HDL, total protein, albumin, lactate dehydrogenase (LDH), urea, creatinine, creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), vitamin C, lipid peroxidation product malondialdehyde (MDA) and protein-bound carbonyls.

Liver, muscle, heart and brain were removed, blotted and weighed and 200 g kg−1 homogenate were prepared in ice-cold 15.5 g L−1 KCL in 0.05 mol L−1 Tris buffer (pH 7.4) using a homogenizer. Portions of organs homogenate were processed for measuring the levels of protein, vitamin C, vitamin E, Glutathione (GSH), MDA and protein-bound carbonyls.

BIOCHEMICAL PARAMETERS

Total protein content was measured in blood and organs by following the method of Lowry et al. (Lowry et al., 1951). Glucose, total cholesterol, HDL, LDH, urea, creatinine, CK, AST, ALT, ALP and GGT were measured on Roche/Hitachi Cobas c systems (Integra 400 Plus), Germany by using enzymatic colorimetric methods.

ANTIOXIDANT STATUS BIOMARKERS

Vitamin C concentration was measured by Omaye et al. method (Omaye et al., 1979), after reaction with 2,4-dinitrophenylhydrazine at 520nm. Vitamin E was measured by HPLC using a fluorescence detector with excitation at 205 nm and emission at 340 nm (Hatam and Kayden, 1979). Glutathione was measured spectrophotometrically at 412 nm after reaction with dithionitrobenzoic acid (Sedlack and Lindsay, 1968).

OXIDATIVE DAMAGE BIOMARKERS

MDA

The concentration of the lipid peroxidation product MDA was measured by using the modified procedure of Li and Chow (Li and Chow, 2004). The reaction mixture was extracted with isobutanol and the fluorescence intensity was measured with excitation at 515 nm and emission at 550 nm using a spectrofluorometer. 1,1,3,3-Tetramethoxypropane was used as standard.

PROTEIN-BOUND CARBONYLS

The content of protein-bound carbonyls, which is used to assess the extent of protein oxidation, was determined spectrophotometrically at 530 nm by the 2,4-dinitrophenylhydrazine method of Levine et al. (Levine et al., 1990).

HISTOPATHOLOGY

Following different organ procurement, tissue fragments were placed in 10% buffered formalin for 8 hours. Manual tissue processing involved dehydration by using ethyl alcohol, clearing by using xylene and infiltration by paraffin. Four-micrometer sections were obtained from paraffin blocks and stained with hematoxylin and eosin. Fragments of liver, muscle, heart and brain were prepared.

STATISTICAL ANALYSIS

Means±s.e. were calculated. Data were subjected to analysis of variance to determine the significance ($P < 0.05$) of main effects, followed by Tukey's multiple comparison test for significance of differences.

RESULTS AND DISCUSSION

GROWTH OF RATS

As shown in table 1, over the period of 13 weeks, the growth rate was higher in DSE1 and DSE2 groups compared to the control with, however, acceptable final weights of 362.44 ± 22.26 and 351.33 ± 37.00g in DSE1 and DSE2 groups, respectively. The results in the literature are mixed. Several other studies in rat reported significant increases in animal weight with date seed-supplemented diets (Hussein et al., 1998; Vandepopuliere et al., 1995;Ali et al., 1999; Elgasim et al., 1995), whereas others observed no weight change (Habib and Ibrahim, 2011; Aldhaheri et al., 2004). The discrepancies in the results among different studies are possibly due to several factors, including differences in the species of experimental animals used, the amount of date seeds fed and the duration of the feeding period.

BLOOD BIOCHEMICAL PARAMETERS

Biochemical parameters measured in blood are shown in Table 1. Slight changes were observed in DSE2 group compared to control group but values remained in accordance with values reported in trials considering effects of phenolic compounds in rats and reference ranges established in rat (Azorin-Ortuno *et al.*, 2008; Cerda et al., 2003; Niho et al., 2001; Ihedioha et al., 2013). Nonetheless, the results globally support the absence of any inflammatory process, injury or dysfunction.

A higher blood glucose level was observed in DSE2 group compared to the control group (p=0.02). There is no clear explanation for this result since rather hypoglycemic effects, even after meal, have been largely reported so far for diverse dietary flavonoids, which are the most abundant polyphenol components found in DSE (Babu et al., 2013).

Protein and albumin levels were slightly lower in DSE2 group compared to control group but do not go below the lower reference values of 5.2g/l for protein and 3.0g/l for albumin, reported by Azorin-Ortuno et al. (Azorin-Ortuno et al., 2008). The absence of abnormally

low albumin is in favor of the nonexistence of any inflammatory processes in the animals.

Regarding total cholesterol and HDL cholesterol levels, both were reduced in DSE2 group compared to control, but were in accordance with reference values recently established by Ihedioha et al. (Ihedioha et al., 2013). DSE1 and DSE2 differed only for total cholesterol level, which was lower in DSE2 (1.47 ± 0.26 mmol/l) than in DSE1 (1.76 ± 0.19 mmol/l). Further studies are warranted to investigate the underling mechanism(s). It is possible that date seed extract exerts an inhibitory effect on HMG CoA Reductase. Even though, cholesterol values in rats are typically about one-third those of humans (Meyer and Harvey, 1998), and the lipoprotein system differs in its functioning between rat and human (Oschry and Eisenberg, 1982), these results are compatible with the absence of any adverse lipid system disturbance in the liver.

Table 1: **Weight and blood biochemical parameters in rat groups**

	Control (n=5)	**Date seed extract 240mg/kg (n=9)**	**Date seed extract 480mg/kg 2 (n=9)**
Growth rate (g/day)	2.56 ± 0.41	3.23 ± 0.30 [a]	3.13 ± 0.46 [a]
Glucose (mmol/l)	7.74 ± 0.79	8.99 ± 0.36	10.65 ± 0.76 [a]
Protein (g/l)	66.60 ± 1.93	64.18 ± 0.93	61.17 ± 1.32 [a]
Albumin (g/l)	41.38 ± 0.99	40.51 ± 0.68	37.85 ± 0.64 [a,b]
Cholesterol (mmol/l)	1.78 ± 0.05	1.76 ± 0.06	1.47 ± 0.09 [a, b]
HDL (mmol/l)	1.09 ± 0.06	0.92 ± 0.04	0.84 ± 0.06 [a]

Means±s.e. are presented. Tukey test was performed to compare rat groups. Statistical significance was set at $p \leq 0.05$.
[a]Statistically significant difference between date seed extract group and control group; [b]Statistically significant difference between date seed extract groups

TISSUE FUNCTION

LDH is usually considered as a good indicator of tissue injury and is found in a greater amount in muscle tissue (Brancaccio et al., 2010). In this study, LDH was lower in DSE1 and DSE2 groups compared to control (Table 2). This supports the absence of any tissue damage or injury in the animals. It can be noticed that the LDH reduction is even greater in DSE2 compared to DSE1 group, suggesting a dose-dependent effect of DSE on LDH. Similar results have already been observed in a study by using date seed powder supplemented diet in rat for 30 days (Habib and Ibrahim, 2011).

Similarly, CK, which is an *in situ* ATP regenerator through the conversion of creatine into phosphocreatine, is particularly abundant in tissues consuming ATP rapidly like skeletal and heart muscle. High serum CK levels are related to tissue damages (Brancaccio et al., 2010), and in this study, CK was significantly decreased in both DSE1 and DSE2 fed rats.

Urea and creatinine are both indicators of kidney function. Creatinine better reflects glomerular function since urea can be influenced by other factors like dehydration. Urea did not differ between the three groups. By contrast, creatinine tended to be lower in DSE2 group compared to control, staying within the values observed in previous studies in rats (Azorin-Ortuno et al., 2008; Cerda et al., 2003; Niho et al., 2001; Ihedioha et al., 2013). Increased levels of creatinine indicate kidney failure. Therefore, these results are rather in favor of a more efficient renal function with date seed extract.

Even though, ALT, AST, ALT and GGT are found in all tissues, they are more concentrated in certain organs, especially in liver. The detection of elevated levels of these enzymes released from tissues into the bloodstream is usually indicative of increased tissue injury. This is commonly associated with certain disease states or exposure to some toxic compounds. The assessment of these enzymes was used to detect liver tissue damage. ALT and AST are recognized as indictors of hepatocyte integrity and ALP and GGT as indicator of cholestasis. In liver, compared to other organs, a greater increase of ALT is needed to suspect any hepatocellular damage whereas slight muscle damage has been associated with a 2-3 fold increase (Meyer and Harvey, 1998). In this study, ALT and AST levels were slightly high compared to normal ranges reported by Azorin-Ortuno et al. (Azorin-Ortuno et al., 2008). These high values may be related to hemolysis. As shown in Table 2, ALP is not significantly lowered in DSE groups compared to control. However, a significant decrease has been observed for ALT and GGT in DSE2 and for AST in both DSE1 and DSE2 groups, to a greater extent in DSE2 than in DSE1.

GGT was globally low in all the animals. Azorin-Ortuno et al. (Azorin-Ortuno et al., 2008) have reported similar low levels. Recently, it has been shown that GGT was of a limited utility for diagnosis of biliary injury in rat, and especially in case of no hepatocellular necrosis (Enmulat et al., 2010). Since GGT was significantly lowered in DSE2 compared to control, this does not only support the lack of any biliary injury, but also a protective effect of date seed extract.

ANTIOXIDANT STATUS AND OXIDATIVE DAMAGES

Globally, a stronger antioxidant defense system is noticed with DSE in serum and organs (Table 3) as well as a lower occurrence of oxidative damages (Figure 1).

Vitamin C is a water-soluble compound and a powerful antioxidant. It can donate a hydrogen atom and form a relatively stable ascorbyl free radical and hence play

the role of free radical scavenger. Similarly, vitamin E, which is the major lipid-soluble antioxidant in the cell antioxidant defense system can act as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues. Vitamin E reacts with free radicals to form a tocopheryl radical, which will then be reduced by a hydrogen donor and thus return to its reduced state. The major biologic role of vitamin E is to protect polyunsaturated fatty acids and other components of cell membranes and low-density lipoprotein from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation (Traber and Stevens, 2011).

Based on the measurement of these two antioxidant components, in serum and organs, a strengthened antioxidant status is suggested by the consumption of DSE, especially DSE2.

Table 2: Biomarkers of tissue function in rat groups

	Control (n=5)	Date seed extract 240mg/kg (n=9)	Date seed extract 480mg/kg 2 (n=9)
Urea (mmol/l)	4.96 ± 0.24	4.66 ± 0.17	4.33 ± 0.20
Creatinine (mmol/l)	35.58 ± 1.05	31.00 ± 1.59	28.70 ± 1.76 [a]
LDH (IU/l)	3690.60 ± 212.57	2710.22 ± 168.96 [a]	2023.76 ± 127.34 [a, b]
Liver protein (g/l)	17.26 ± 0.60	19.98 ± 0.49	21.07 ± 0.93 [a]
ALT (IU/l)	62.60 ± 10.67	43.67 ± 1.52	35.63 ± 4.50 [a]
ALP (IU/l)	97.05 ± 7.21	78.64 ± 4.40	75.88 ± 5.09
AST (IU/l)	337.60 ± 41.38	251.22 ± 14.19 [a]	194.71 ± 12.66 [a]
GGT (IU/l)	5.75 ± 1.25	3.78 ± 0.36	3.00 ± 0.53 [a]
Creatine Kinase (IU/l)	11701.25 ± 846.36	7561.50 ± 846.22 [a]	7067.71 ± 592.22 [a]

Means±s.e. are presented. Tukey test was performed to compare rat groups. Statistical significance was set at $p \leq 0.05$. [a]Statistically significant difference between date seed extract group and control group; [b]Statistically significant difference between date seed extract groups.

Except in liver and for DSE1, where vitamin C was significantly higher, vitamin C level did not differ between the three groups, which is in accordance with the previous results published by Habib and Ibrahim, by using date seed powder supplemented diet in rat (Habib and Ibrahim, 2011). This lack of significant increase of vitamin C level in serum and organs, may be related to the fact that rat, contrary to human, is able to newly synthesize vitamin C. Recycling process of vitamin C would hence be less essential to maintain the antioxidant function (Michels and Frei, 2013).

A clear global trend to increase can be observed for vitamin E in serum, liver and muscle. However, it is statistically significant in muscle only, in both DSE1 and DSE2 groups. This may be related to the wide variability noted for this parameter and the sensitivity of the method. Vitamin E can be obtained only from diet. After vitamin E ingestion, a rapid increase has been reported in plasma but also in the main storage organs like muscle. In addition, fast recycling mechanisms to maintain blood vitamin E in the reduced state exist. Oxidative status-dependent exchange between plasma and muscle and recycling mechanism has been described. It means that the level of vitamin E necessary for optimal antioxidant activity is not absolute because the turnover will change in response to oxidant pressure (Traber et al., 1993). This suggests that in the current environmental pro-oxidant situation, the incorporation of DSE in the diet could contribute to a more efficient trafficking of vitamin E towards particularly metabolically active organs like muscle. No difference in vitamin E level was reported by Habib and Ibrahim (Habib and Ibrahim, 2011) and this may be related to the shorter duration of their study and the use of date seed powder which may make phenolic compounds less bioavailable.

The increase in Vitamin E level could also be related to the greater level of GSH, which is the reduced form of glutathione, in liver, muscle and heart. Indeed, GSH is known to interact with Vitamin E to maintain it into its reduced form (Scholz et al., 1989). If GSH is increased in liver, muscle and heart, reflecting hence a strengthened antioxidant defense system with the consumption of DSE, it is not the case in the brain. Considering the important functions of GSH, not only in the antioxidant defense system but also in metabolic processes, it is not surprising that the level of GSH is tightly regulated in organs. This is particularly the case in the brain. If the brain is one of the organs with the greatest production of reactive oxygen species it is also the organ with the lowest level of GSH due to the potential toxicity of the substrates which are used to synthesize it (Smeyne and Smeyne, 2013;Lushchak, 2012). Production and used of GSH are strictly regulated to maintain this low level.

As shown in Figure 1, products of oxidation, especially those of lipid oxidation, are significantly reduced in serum and organs.

In brain, protein-bound carbonyls are significantly reduced in the group having consumed DSE at a dose of 480mg of DSE/kg diet. This indicates that DSE is related to a reduced protein oxidation process in the brain especially. Since protein oxidation has been related to brain neuro-degenerative disorders as well as a faster aging process, this suggests that DSE intake could contribute to protect against them.

Regarding MDA, it is reduced in serum and all organs. It is statistically significant in liver, muscle and brain, whatever the concentration of DSE in the diet. These results are similar to the observations made in serum and liver by Habib and Ibrahim in rats supplemented with date

seed powder for 30 days (Habib and Ibrahim, 2011). In heart, only DSE2 is associated with a significant decrease of MDA. These results demonstrate a clear antioxidant status improvement with DSE in blood and organ tissues, through a strengthened antioxidative defense system and a limitation of oxidative damages. This indicates that DSE could have a double impact: one on the antioxidant system, with an improvement of vitamin E and GSH levels as described above and another with the reduction of oxidative damages. This latter could be explained by a potential effect on the body repair systems, which are involved in the protection of the macromolecules.

Since, it is well-recognized that phenolic compounds, like those which were identified in date seed extract (Habib et al., 2014), exert radical scavenging activity and protect against lipid and protein oxidative damages (Sies, 1997), it can reasonably be assumed that the health benefits of DSE are related to its polyphenols.

Interestingly, DSE exerted positive effects even in the brain. This is of a great importance since brain is one of the tissues with the lowest antioxidant defense system, and oxidative damages are likely to lead to major neuro-degenerative disorders including Alzheimer and Parkinson's diseases.

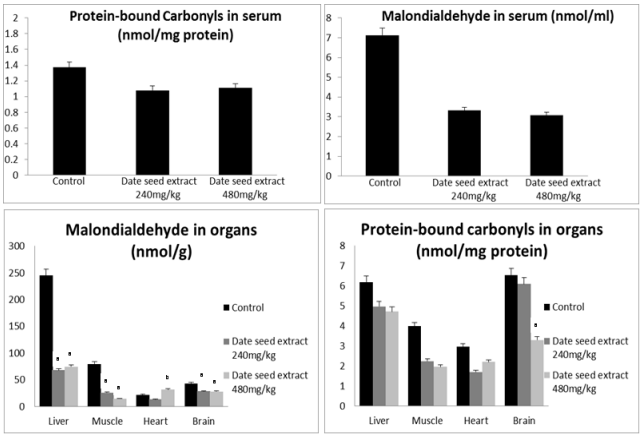

Figure 1: Biomarkers (protein-bound carbonyls and malondialdehyde) of oxidative damages in serum and organs in rat groups

Tukey test was performed to compare rat groups. Statistical significance was set at $p \leq 0.05$. [a]Statistically significant difference between date seed extract group and control group; [b]Statistically significant difference between date seed extract groups.

Table 3: Antioxidant markers (Vitamin C, Vitamin E, and GSH) in serum and organs in rat groups

	Control (n=5)	Date seed extract 240mg/kg (n=9)	Date seed extract 480mg/kg (n=9)
Vitamin C			
Serum (mcg/ml)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Liver (mcg/g)	0.30 ± 0.03	0.62 ± 0.09 [a]	0.40 ± 0.06
Muscle (mcg/g)	8.74 ± 1.02	10.02 ± 1.03	8.02 ± 0.65
Heart (mcg/g)	26.20 ± 1.34	20.95 ± 1.11	19.01 ± 1.04
Brain (mcg/g)	63.50 ± 3.06	63.87 ± 2.01	56.81 ± 2.20
Vitamin E			
Serum (mcg/ml)	1.66 ± 0.43	2.14 ± 0.45	2.25 ± 0.49
Liver (mcg/g)	23.94 ± 2.67	37.03 ± 8.74	51.84 ± 14.26
Muscle (mcg/g)	5.52 ± 0.73	7.96 ± 0.44 [a]	8.69 ± 0.28 [a]
GSH			
Liver (nmol/g)	568.80 ± 5.71	558.19 ± 13.37	645.00 ± 26.06 [b]
Muscle (nmol/g)	159.91 ± 18.14	400.80 ± 16.28 [a]	414.41 ± 12.39 [a]
Heart (nmol/g)	475.98 ± 12.04	662.29 ± 18.98 [a]	624.29 ± 30.80 [a]
Brain (nmol/g)	487.16 ± 20.38	469.99 ± 7.90	498.27 ± 18.88

Means±s.e. are presented. Tukey test was performed to compare rat groups. Statistical significance was set at $p \leq 0.05$. [a]Statistically significant difference between date seed extract group and control group; [b]Statistically significant difference between date seed extract groups.

HISTOPATHOLOGY

Liver, muscle, heart and brain samples of control rats, rats from DSE1 and DSE2 showed all a normal appearance (Figure 2). No change related to ischemia was detected.

These observations confirm the absence of any changes related to the oral consumption of DSE by rats.

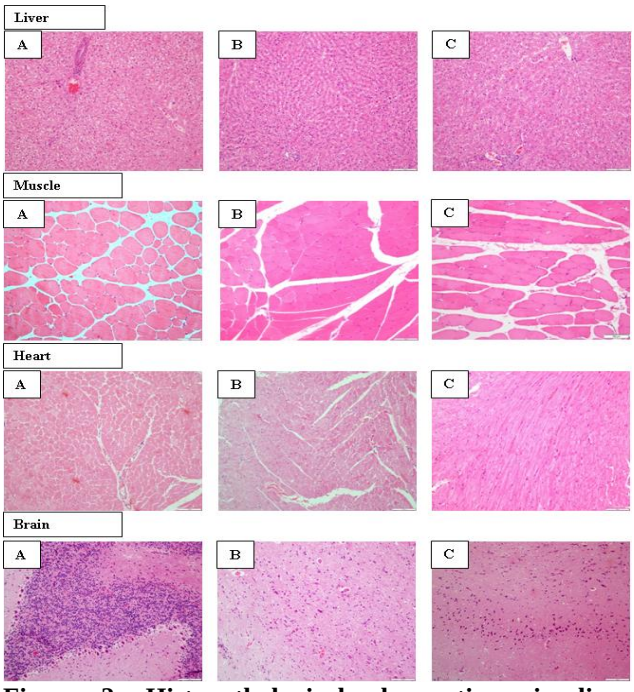

Figure 2: Histopathological observations in liver, muscle, heart and brain.
Hematoxylin and eosin×20. (A) Control group, (B) date seed extract 240mg/kg diet, (C) date seed extract 480mg/kg diet.

CONCLUSION

Our data indicate that supplementing rats with DSE did not alter tissue function as shown by albumin, urea, creatinine, CK, ALT, ALP, AST, GGT, LDH and histopathological results. Furthermore, DSE protected against oxidative damages, through its antioxidant compounds and by, potentially, strengthening the antioxidant defense system. DSE appears as a strong candidate to counteract the negative effects of the environment on the oxidative balance. These conclusions have to be confirmed in humans for a potential use of DSE as functional ingredient. In addition, the mechanisms underlying these protective effects remain to be described.

ACKNOWLEDGMENTS

This research project received the United Arab Emirates Research Grant in 2012.

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