

Effect of date fruit supplemented diet on serum lipidemic and oxidative stress biomarkers in rodent experimental modelling

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Abstract

The present work was designed to explore the functionality of Pakistani date cultivar (Zahidi) against atherogenic diet induced oxidative stress. The bioefficacy assessment of Zahidi date fruit and extract was carried out via model feeding trial. The rats (Sprague Dawley) were divided into two broad categories; one fed with normal diet, whereas the other was fed with atherogenic diet to induce oxidative stress. The formulated groups were G₁ (normal diet), G₂ (date fruit + normal diet), G₃ (date extract + normal diet), G₄ (atherogenic diet), G₅ (date fruit + atherogenic diet) and G₆ (date extract + atherogenic diet). The results showed that date fruit reduced 3.65% serum cholesterol in normal rats, and 15.14% in atherogenic rats, while, the date extract treatment resulted in 4.49 and 18.55% reduction in normal and atherogenic rats, respectively. Low-density lipoprotein (LDL) cholesterol reduced by 8.66 and 11.55% in date fruit and date extract fed normal rats, respectively, while 21.05 and 25.98% reductions were noted in the atherogenic groups fed with date fruit and date extract, respectively. To assess the extent of cardiac risk in the subjects, several atherogenic ratios were calculated based on the serum lipid parameters. The analysis showed that atherogenic diet groups were at higher risk than the normal ones, and that date fruit and extract containing functional diets effectively ameliorated the elevated risk ratios. The date fruit and extract-based diets intensified serum superoxide dismutase (SOD) and catalase (CAT) levels throughout the study up to 29.05 and 27.99%, respectively. Date fruit effectively lowered lipid peroxidation by 13.64 and 33.67% in normal and atherogenic rats, respectively. Conclusively, date fruit and extract treatment proved effectual in modulating the serum lipid profile and effectively restored the SOD and CAT levels alongside reducing the lipid peroxidation.

Keywords

Zahidi date fruit
serum cholesterol
lipid peroxidation
endogenous enzymes
atherogenic diet
atherogenic index

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Introduction

The introduction of diet-based therapeutic interventions around the globe are aimed to utilise foods and their constituents as remedial agents against prevailing metabolic ailments. The role of preventive approaches in health maintenance resulted in the development of novel health care practices that are supported by strong scientific evidences (Goldberg, 2012; Olaiya *et al.*, 2016). This leads to a paradigm shift from the intervention-oriented and technology-driven outlooks of the previous era to more advanced

prophylactic and molecular-based tactics (Joseph *et al.*, 2016). The diet-health linkages and their economic and social implications are gaining wide public acceptance globally. The knowledge about the health-benefiting role of foods and their components has grown immensely over the past few decades (Pathak, 2009). This has resulted in increased consumers' desire and demand for the healthy diets. Hence, natural foods such as black cumin, garlic, ginger and flaxseed that exert affirmative effects on the human health are being used (Pathak, 2009). The functional foods, alongside providing basic

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nutrition, contain an optimal mix of biologically active constituents often termed as nutraceuticals. These are involved in improving and protecting the physiological functionality (Goldberg, 2012; Lu and Yen, 2015).

Numerous epidemiological investigations have suggested an inverse association between the diseases of affluence, such as cardiovascular ailments, hepatic disorders, hypercholesterolemia, diabetes and oncogenesis and the consumption of fruits, vegetables and herbs (Awan *et al.*, 2019). These commodities contain a myriad of phytochemicals that pose positive effects on the human health. Date palm (*Phoenix dactylifera* L.) belongs to the Arecaceae family and grows in the hot arid regions of the world. The fruit is savoured for its sweet taste and fleshy mouth feel. The fruit pulp contains phenolics, flavonoids, carotenoids, sterols, anthocyanins and procyanidins (Al-Farsi *et al.*, 2005). Various phytochemicals belonging to hydroxybenzoic acid and hydroxycinnamic acid category are present in the date fruit that are responsible for its antioxidant and disease ameliorating characteristics. Date fruits contain phenolics such as cinnamic, sinapic, ferulic, vanillic, syringic, caffeic, chlorogenic, coumaric and protocatechuic acids and their derivatives. Dactyliferic acid; a derivative of hydroxycinnamic acid, has also been identified (Mansouri *et al.*, 2005; Borochoy-Neori *et al.*, 2013; Habib *et al.*, 2014). Among the flavonoids; glycosides quercetin, luteolin, methyl quercetin and methyl luteolin are present. Studies also reveal the presence of catechins, epicatechins and anthocyanins (Al-Farsi *et al.*, 2005; Rock *et al.*, 2009; Habib *et al.*, 2014). However, the presence of these compounds and their quantity depend on the fruit variety and type, maturation stage and the prevailing environmental conditions (Baliga *et al.*, 2011). Owing to the beneficial effects of these compounds on the physiological system, three Pakistani date varieties (Dhaki, Zahidi and Aseel) were screened (Awan *et al.*, 2018). Pakistan is among the largest date producing countries in the world. However, the produced fruits are not fully utilised, and have not yet been explored for their nutraceutical worth. The cultivars were selected based on their quality as well as consumption in the country.

It was found in the preliminary studies that Zahidi dates possess ample amount of nutraceuticals with higher quantities of gallic acid, caffeic acid, EGCG and kaempferol. Therefore, the present work was aimed at exploring the restorative potential of Zahidi dates against high fat diet induced oxidative stress. It is hypothesised that Zahidi dates consumption could modulate lipid biomarkers in atherogenic rats.

Materials and Methods

Materials, chemicals and reagents

Zahidi dates were purchased from a local market in Faisalabad, Pakistan. The sample selection was random and based on certain quality characteristics (i.e., colour uniformity, size, shape and being free from abrasion). The samples were graded and washed before refrigerated at 4°C for further analyses. Male Sprague Dawley rats were housed in the Animal Room of National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. Chemicals used in the analyses were ethylenediamine tetraacetic acid (EDTA), L-methionine, triton X, nitroblue tetrazolium, riboflavin, hydrogen peroxide solution, trichloroacetic acid (TCA) and thiobarbituric acid (TBA). All the chemicals were of analytical grade.

Sample preparation

The date fruit samples were cleaned and pitted. The fruit pulp was minced using an electric mincer (Philips, HR2710) and the samples were kept in polyethylene bags at 4°C for further analyses. Date extract was prepared by dissolving date paste into water (1:3 w/v). The obtained mixture was kept at 4°C for 48 h. Afterwards, it was shaken at 400 rpm for 45 min, followed by centrifugation at 5,000 rpm at 4°C for 45 min using centrifugal machine (M-3k30, Sigma, Germany). Supernatant was collected and used in the in the bioefficacy studies (El Arem *et al.*, 2014).

Bioefficacy studies

To explore the functionality of dates against diet-related disorders with special reference to lipidemic and oxidative stress, a model feeding trial was carried out. Purposely, 60 male Sprague Dawley rats (4 w old) weighing around 120-140 g were placed in the animal room of National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. The rats were housed individually in stainless steel cages in an air-conditioned room at 23 ± 2°C, 55-60% relative humidity and 12-hour light-dark cycle. The animals were acclimatised by feeding basal diet for 1 w. They were fed on specific diets and tap water ad libitum throughout the experimental period.

It was assured that all the feed model trials were conducted in compliance with the relevant institutional laws and guidelines of University of Agriculture, Faisalabad, Pakistan. Moreover, the study plan involving rat experimental trials and dietary supplementation of date fruit/extract

were thoroughly reviewed and approved by the institutional committee(s). The biological efficacy assessment trials were carried out using the diagnostic kits (CHOD-PAP) procured from Sigma-Aldrich (Germany) and Cayman Chemicals (Estonia).

Experimental design

Rats (n = 10) were randomly divided into six groups. G₁ served as control comprising of rats fed with normal diet. In G₂ and G₃ groups, rats were fed with normal diet and functional date fruit and date extract, respectively. In G₄, rats were given atherogenic diet comprising of normal rat chow along with cholesterol (1.5%) and cholic acid (0.5%) to induce oxidative stress. In G₅, rats were fed with atherogenic diet with date fruit, while in G₆, rats were fed with atherogenic diet with date extract (Table 1). The dietary supplementation of date fruit at 20% was used in the case of date fruit while equivalent amount of date extract was added to the diet of the date extract fed rats. After 12 w, the overnight fasted rats were sacrificed, and sera were collected for biomarkers assessment.

Table 1. Bioefficacy plan.

Diet	Group	Dietary Module
Normal Diet	G ₁	Normal diet
	G ₂	Date fruit + normal diet
	G ₃	Date extract + normal diet
Atherogenic Diet	G ₄	Atherogenic diet
	G ₅	Date fruit + atherogenic diet
	G ₆	Date extract + atherogenic diet

Serum lipidemic profile

Serum triacylglycerol (TG) and total cholesterol (TC) were determined using the commercially available Fluitest TG (Triglyceride GPO-PAP) and Fluitest Chol (Cholesterin CHOD-PAP) kits (Biocon, Vöhl-Marienhagen, Germany), respectively. High density lipoproteins (HDL) were ascertained by HDL precipitant method using commercially available Ecoline kits (Merck, Germany). The analysis was performed on Semi Automated Clinical Chemistry Analyser (Microlab 300, Merck, Netherlands). Friedewald formula as described by Friedewald (Friedewald *et al.*, 1972) was used for calculating the low density lipoproteins (LDL), very low density lipoprotein (VLDL) and non-high density lipoprotein (nHDL):-

$$\text{LDL} = \text{Total cholesterol} - \text{HDL} - \text{VLDL} \quad (\text{I})$$

$$\text{VLDL} = \frac{\text{Triglycerides}}{5} \quad (\text{II})$$

$$\text{nHDL} = \text{Total cholesterol} - \text{HDL} \quad (\text{III})$$

Atherogenic ratios

Lipoprotein risk ratios including atherogenic index of plasma (AIP), atherogenic coefficient (AC), Castelli risk index (CRI) I and II were calculated using mathematical expressions (Jamil and Siddiq, 2012; Hassan *et al.*, 2015) as follows:

$$\text{AIP} = \frac{(\log \text{ TG})}{\text{HDL}} \quad (\text{IV})$$

$$\text{AC} = \frac{\text{TC} - \text{HDL}}{\text{HDL}} \quad (\text{V})$$

$$\text{CRI(I)} = \frac{\text{TC}}{\text{HDL}} \quad (\text{VI})$$

$$\text{CRI(II)} = \frac{\text{LDL}}{\text{HDL}} \quad (\text{VII})$$

Superoxide dismutase activity

The superoxide dismutase activity was spectrophotometrically assayed as described by Zargar *et al.* (2015). The enzyme activity was determined by mixing 250 μL phosphate buffer (50 mM, pH 7.8), containing 0.3 mM EDTA, 100 μL L-methionine, 100 μL triton X, 50 μL nitroblue tetrazolium and 400 μL distilled water. Then, 50 μL serum sample was added to the reaction mixture followed by the addition of 50 μL riboflavin. Later, the tubes were illuminated under UV light for 20 min. A control tube, in which the sample was replaced by a buffer, was also analysed and the absorbance was measured at 560 nm. One unit of SOD represents the amounts of enzymes that required to inhibit the rate of NBT oxidation by 50% at 25°C. The SOD activity was calculated in unit/mL.

Catalase activity

The catalase activity in the rat sera was measured following the method described by Bulucu *et al.* (2008). The principle is based on the rate constant determination or the H₂O₂ decomposition rate at 240 nm. Briefly, 20 μL sample was added to a cuvette containing 780 μL phosphate buffer (pH 7.5). The reaction was initiated by the addition of 100 μL freshly prepared 500 mM hydrogen peroxide solution. The H₂O₂ disappearance was kinetically monitored at 240 nm for 1 min at 25°C. Enzyme content was calculated using an extinction coefficient of 0.0436 mM⁻¹cm⁻¹. One unit of activity is equal to 1 μmol of H₂O₂ destroyed/min. The activity was expressed in unit/mL serum.

Lipid peroxidation

The lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) in serum using an established protocol of Li *et al.* (2000). For each sample, 0.5 mL serum was transferred into 3.0 mL 20% TCA solution containing 0.5% TBA. After vortexing, each sample mixture was incubated in a 60°C water bath for 30 min, cooled in ice water bath and allowed to rest for 30 min. After cooling, each sample mixture was centrifuged at 10°C at 3,000 rpm for 15 min. The absorbance of the upper organic layer of the centrifuged solution was measured at 532 nm and 600 nm with a microplate reader. The final unit of TBARS value was calculated and expressed as nM/mL in form of MDA (malondialdehyde) content.

Statistical analysis

The data obtained for each parameter were subjected to statistical analysis to determine the level of significance. Completely randomised design (CRD) was applied using Statistical Package (Statistix 8.1) following the principles outlined by Mason *et al.* (2003). Significant ranges were further compared by post-hoc Tukey's HSD test.

Results and discussion

Serum lipidemic profile

In the present work, the effect of date fruit supplemented diet was assessed on normal and atherogenic rats. The oxidative stress was induced using high fat atherogenic diet; therefore, the effect on the serum lipidemic parameters was specially focused. Cholesterol, HDL and VLDL showed non-significant behaviour with respect to treatments in normal diet fed groups. However, significant variations were recorded in the atherogenic diet fed ones. The serum triglycerides, LDL and nHDL were significantly affected by the treatments during the study period in all groups.

Cholesterol; a hydrophilic lipid, is a precursor of several hormones, bile acids and vitamin D. It is required by the body from exogenous sources and is endogenously synthesised through mevalonate pathway. The amount is regulated through feedback control mechanisms (Rafieian-Kopaei *et al.*, 2014). Means for normal diet fed groups i.e. G₁, G₂, G₃ depicted non-significant decrease in serum cholesterol level from 80.57 ± 2.49 mg/dL in the control group to 77.63 ± 2.64 mg/dL in the date fruit fed group and 76.95 ± 3.31 mg/dL in the group provided with date extract containing diet. In the case of atherogenic rats, significant decline in the serum cholesterol level was noted. The diet elevated the cholesterol levels

to 168.34 ± 5.94 mg/dL that decreased to 142.86 ± 4.57 and 137.11 ± 4.94 mg/dL in G₅ (date fruit + atherogenic diet) and G₆ (date extract + atherogenic diet), respectively.

The serum triacylglycerol also significantly decreased in normal as well as stressed rats. The triacylglycerol level in G₁ (normal diet) was 64.12 ± 2.05 mg/dL that reduced to 61.74 ± 2.34 mg/dL in G₂ (date fruit + normal diet) and 60.88 ± 2.62 mg/dL in G₃ (date extract + normal diet). In the atherogenic diet fed rats, maximum triacylglycerol level was found in G₄ as 128.15 ± 4.61 mg/dL that reduced in G₅ (date fruit + atherogenic diet) and G₆ (date extract + atherogenic diet) to 112.74 ± 4.12 and 109.56 ± 3.73 mg/dL, respectively.

Low-density lipoprotein cholesterol (LDL-c) is an imperative risk factor for cardiovascular disparities and primary target for CVD risk reduction strategies (Hoogeveen *et al.*, 2014). The results achieved in the present work revealed significant variations pertaining to the LDL level in all groups. The means for normal diet fed groups presented decreasing trend for LDL in G₁ (35.86 ± 1.11 mg/dL) followed by G₂ (32.75 ± 1.05 mg/dL) and G₃ (31.71 ± 1.14 mg/dL). Similarly, in the rats fed with atherogenic diet, G₄, showed highest LDL (116.28 ± 3.63 mg/dL) that gradually reduced in G₅ (91.80 ± 2.97 mg/dL) and G₆ (86.07 ± 2.84 mg/dL), respectively.

High density lipoprotein cholesterol is considered as good cholesterol and is found to exert prophylactic potential against coronary heart disease possibly through reverse cholesterol transport mechanism and by reducing the LDL associated oxidative stress (Tehrani *et al.*, 2013). HDL explicated non-significant differences for normal diet fed rats whereas, the effect was momentous in the rats fed with atherogenic diet. Means relating to HDL in normal diet fed animals showed that treatments did not considerably alter HDL. Nevertheless, values for G₁, G₂ and G₃ groups were 31.89 ± 0.98, 32.53 ± 1.20 and 33.06 ± 1.06 mg/dL, respectively. Nevertheless, the mean HDL concentration for G₄ was 26.43 ± 0.83 mg/dL that improved to 28.51 ± 1.05 mg/dL in G₅ and 29.13 ± 1.03 mg/dL in G₆.

In the normal diet fed rats, means for VLDL in G₁, G₂ and G₃ groups were 12.82 ± 0.40, 12.35 ± 0.53 and 12.18 ± 0.55 mg/dL respectively. In the atherogenic rats, G₄ showed highest VLDL level at 25.63 ± 0.90 mg/dL, followed by 22.55 ± 0.86 and 21.91 ± 0.75 mg/dL in G₅ and G₆, respectively.

Non-HDL cholesterol (nHDL) is a predictor of CVD risk indicating the content of atherogenic apolipoprotein B containing lipoproteins (LDLs, VLDLs, and IDLs). The nHDL levels also significantly

varied as a function of treatments in normal and oxidative stressed rats. In normal rats, mean values for nHDL were 48.68 ± 1.51 , 45.10 ± 1.62 and 43.89 ± 1.40 mg/dL for G₁, G₂ and G₃ groups, respectively. Likewise, in the atherogenic diet fed groups, the mean for n-HDL in G₄ was 141.91 ± 4.26 mg/dL that reduced significantly to 114.35 ± 3.89 mg/dL in G₆ and 107.98 ± 3.73 mg/dL in G₅.

Overall, date fruit reduced 3.65% serum cholesterol in normal rats, whereas 15.14% in atherogenic rats, while, the date extract treatment resulted in 4.49 and 18.55% cholesterol reduction in normal and atherogenic rats, respectively. Likewise, triacylglycerol level decreased by 3.71 and 12.02% by date fruit supplementation in normal and atherogenic rats, respectively. The percent decrease in date extract fed groups were 5.05 and 14.51% in normal and stressed rats, respectively. LDL cholesterol reduced by 8.66 and 11.55% in date fruit and extract fed normal rats, respectively. Whilst, 21.05 and 25.98% reduction were noted in the atherogenic groups fed with date fruit and extract containing diets, respectively.

High fat diets have been used for decades to model dyslipidemia, obesity and insulin resistance in rats. Studies validate that such dietary regimens result in metabolic syndrome that closely resembles the human metabolic dysfunction and may extend to more severe cardiac complications (Buettner *et al.*, 2006). High dietary cholesterol increases the serum and tissue lipidemic markers that are considered as the major risk factors associated with atherosclerosis. The hepatic cholesterogenesis is suppressed due to excess dietary cholesterol and leads to deposition in the system. In the present work, 1.5% cholesterol and 0.5% cholic acid were sufficient to induce atherogenic conditions, particularly by elevating the lipid levels (Bravo *et al.*, 2014).

The present findings pertaining to the lipid lowering potential of date fruit supports the earlier outcomes of Al-Yahya *et al.* (2016). The authors reported the dyslipidemic potential of Ajwa date fruit variety in isoproterenol (ISP)-induced cardiotoxic rodent model. The increase in lipid profile as a result of ISP was significantly ameliorated by the Ajwa extracts in a dose dependent manner. The possible cholesterol lowering mechanism suggested by the authors focused on the high fiber content in the fruit along with the presence of bioactives and selenium.

In another study, Khalas date pulp was administered on hamsters along with normal and cholesterol containing diets for 13 weeks. Their findings are in accordance with the current results. In the case of normal diet fed rats, no significant effect was observed in cholesterol, LDL, TG and HDL levels. However, the cholesterol containing diet considerably raised the lipid profile, whereas date supplemented diet proved effective in modulating the altered lipid profile (Alsaif *et al.*, 2007). The findings of Rock *et al.* (2009) are also in close agreement with the present work. They summarised their study by declaring date fruit as an anti-atherogenic nutrient because its consumption significantly decreased the serum triacylglycerol levels substantially by 8 to 15%, alongside decreasing the basal oxidative stress up to 33% in healthy subjects for four weeks at 100 g/d dose. They also reported an increase in PON1 activity associated to HDL by 8%. The positive outcomes regarding the anti-atherogenic potential of date supplementation were associated to the presence of catechins and dietary fiber.

The present work also validated the findings of Vembu *et al.* (2012) who deduced that phytochemical constituents of date fruit extract are responsible for preventing high fat diet induced obesity and restores the elevated lipid profile as a result of high

Table 2. Effect of diets on serum lipidemic profile.

Diet	Experimental Group	Serum Lipidemic Parameters (mg/dL)					
		Cholesterol	Triacylglycerol	HDL	LDL	VLDL	nHDL
Normal Diet	G ₁	80.57 ± 2.49^{NS}	$64.12 \pm 2.05^{**}$	31.89 ± 0.98^{NS}	$35.86 \pm 1.11^{**}$	12.82 ± 0.40^{NS}	$48.68 \pm 1.51^{**}$
	G ₂	77.63 ± 2.64^{NS}	$61.74 \pm 2.34^*$	32.53 ± 1.20^{NS}	$32.75 \pm 1.05^*$	12.35 ± 0.53^{NS}	$45.10 \pm 1.62^*$
	G ₃	76.95 ± 3.31^{NS}	$60.88 \pm 2.62^*$	33.06 ± 1.06^{NS}	$31.71 \pm 1.14^*$	12.18 ± 0.55^{NS}	$43.89 \pm 1.40^*$
	F value	2.62^{NS}	4.64^*	1.43^{NS}	22.66^{**}	2.90^{NS}	4.70^*
Atherogenic Diet	G ₄	$168.34 \pm 5.94^{**}$	$128.15 \pm 4.61^{**}$	$26.43 \pm 0.83^*$	$116.28 \pm 3.63^{**}$	$25.63 \pm 0.90^{**}$	$141.91 \pm 4.26^{**}$
	G ₅	$142.86 \pm 4.57^*$	$112.74 \pm 4.12^*$	$28.51 \pm 1.05^*$	$91.80 \pm 2.97^*$	$22.55 \pm 0.86^*$	$114.35 \pm 3.89^*$
	G ₆	$137.11 \pm 4.94^*$	$109.56 \pm 3.73^*$	$29.13 \pm 1.03^{**}$	$86.07 \pm 2.84^*$	$21.91 \pm 0.75^*$	$107.98 \pm 3.73^*$
	F value	49.19^{**}	49.22^{**}	13.17^{**}	99.69^{**}	49.92^{**}	126.75^{**}

Data are means \pm SD ($n = 10$); One-way ANOVA followed by Tukey's HSD multiple comparison tests; * = significant; ** = highly significant; NS = non-significant; G₁ = normal diet; G₂ = date fruit + normal diet; G₃ = date extract + normal diet; G₄ = atherogenic diet; G₅ = date fruit + atherogenic diet; G₆ = date extract + atherogenic diet.

dietary fat intake. Plant sterols present in the fruit extract could possibly pose beneficial effect against hypercholesterolemic conditions. The phytosterols reduce the cholesterol absorption by increasing the faecal steroid excretion, thereby reducing the lipid contents (Gylling *et al.*, 2014). Moreover, the flavonoids may increase the activity of lecithin acyltransferase that plays an imperative role in regulating the blood lipids. It is an anti-atherogenic enzyme that causes esterification of free cholesterol on HDL particles, thereby facilitating reverse cholesterol transport (You *et al.*, 2008).

Evidences support that the hypercholesterolemic conditions are strongly associated with cardiac ailments (Dawber *et al.*, 2015). Clinical trials have demonstrated that reducing the total cholesterol and LDL by 1% decreases the incidence of coronary heart disease by 1.5% (American Academy of Pediatrics, 1992). The cholesterol lowering mechanism of date fruit particularly Zahidi variety and its extract is most likely to be mediated by the presence of major bioactives such as gallic acid, caffeic acid, EGCG, kaempferol and quercetin.

Gallic acid (3,4,5-trihydroxybenzoic acid) is known to exhibit certain beneficial biological properties. In the studied date variety (Zahidi), gallic acid is present in abundant quantity that may possibly be responsible for its lipid lowering potentials. The study conducted by Hsu and Yen (2007) demonstrates the efficacy of gallic acid against high fat diet-induced dyslipidemic conditions. Gallic acid supplementation at 100 mg/kg B.W. for 10 days reduces the total cholesterol level (18.33%), LDL cholesterol (37.87%) and triacylglycerol (13.26%) whilst, the HDL level increases non-significantly (6.52%), thus indicating strong hypolipidemic potential. Moreover, the serum leptin levels also decrease significantly that is directly associated with the body fat. Leptin may increase the intracellular fatty acids by contributing to hepatic steatosis through altering hepatocyte insulin signalling (Uygun *et al.*, 2000). Hence, the plausible hypocholesterolemic mechanism of gallic acid may focus on modulating the leptin levels by preventing their increase, thereby reducing the body fat content. Moreover, gallic acid inhibits the hepatic cholesterol biosynthesis, stimulates receptor-mediated catabolism of LDL and increases the faecal bile acid secretion. Therefore, it can be inferred that the lipogenesis inhibiting potential of gallic acid is responsible for reducing the triglycerides, LDLs and VLDLs (Kulkarni and Viswanatha Swamy, 2015).

Similarly, several scientific evidences endorse the anti-atherosclerotic potential of EGCG, kaempferol and quercetin (Salvamani *et al.*, 2014). In this vista,

Kong *et al.* (2013) suggested that 30 and 150 mg/kg kaempferol treatment for six and ten weeks prevents high cholesterol induced atherosclerosis in New Zealand rabbits. It decreases the serum lipids and improves the antioxidant ability, besides down-regulating the protein and gene expression of pro-atherogenic biomolecules (MCP-1, E-sel, VCAM-1, ICAM-1). It also reduces the release of IL-1 β and TNF- α . A more mechanistic insight to the cholesterol regulating mechanism of date fruit polyphenols may be described through a docking study conducted by Islam *et al.* (2015). Their research clearly demonstrates that EGCG, kaempferol and quercetin can sterically hinder HMG-CoA binding to substrate by blocking the active site through to the L-domain and cis-loop resulting in substrate cavity, therefore, acting as competitive inhibitors.

Besides, date fruit is rich in dietary fiber. Dietary fiber quantification has not been carried out in the present work, but previous studies suggest that dates are abundant in dietary fiber. The crude fiber analysis shows that the selected variety contains maximum amount. Irrefutable scientific evidences based on cohort studies and meta-analysis positively correlate dietary fiber consumption and reduced risk of CVDs by modifying blood lipid profiles particularly decreasing the LDL cholesterol (Riccioni *et al.*, 2012; Wu *et al.*, 2015).

Atherogenic risk ratios

Dyslipidemic conditions involve overproduction or deficiencies of lipoproteins that result in disorders in lipoprotein metabolism. These lipoproteins are divided into four major classes that include chylomicrons - the triglyceride rich particles, very low-density lipoproteins (VLDL), the cholesterol rich low-density lipoproteins (LDL) and the high-density lipoproteins (HDL). Evidences based insights from the epidemiological, pathological and metabolic studies strongly support the causal relation between atherosclerosis and serum lipoproteins (Aviram, 2010).

Several atherogenic ratios have been developed involving simple lipid tests to assess the peril of cardiovascular disorders. Accordingly, the atherogenic index of plasma (AIP) is based on triacylglycerol and HDL, and the atherogenic coefficient (AC) is based on total cholesterol and HDL. Similarly, Castelli risk index one [CRI (I)] also involves cholesterol and HDL as the determinants of cardiac malfunctioning. Whereas, Castelli risk index II [CRI (II)] is another fraction that involves the independent CVD risk factors i.e. HDL and LDL. These ratios are relied upon due to their significance

in predicting the risk of cardiac stress (Nimmanapalli *et al.*, 2016).

The atherogenic index of plasma reflects the balance between the damaging and restoring lipoproteins i.e. triacylglycerol and high-density lipoproteins. Significant effect of treatment was recorded in normal as well as atherogenic rats. In the case of normal rats, G₁ exhibited 0.30 ± 0.01 AIP value that reduced due to date fruit as well as date extract treatment to 0.28 ± 0.01 in G₂ and 0.27 ± 0.01 in G₃ group. However, concerning the atherogenic rats, considerable increase of 0.69 ± 0.02 was observed in the AIP ratio that decreased in the date fruit treated groups G₅ and G₆ to 0.60 ± 0.01 and 0.58 ± 0.02 , respectively.

The atherogenic coefficient is a ratio between non-HDL and HDL. The present results suggest significant impact of treatments on the AC values. The recorded ratio was higher in normal diet fed rats (G₁) 1.53 ± 0.05 that decreased in the preceding date fruit (G₂) and date extract (G₃) fed groups to 1.39 ± 0.06 and 1.33 ± 0.06 , respectively. Likewise, in the rats fed on atherogenic diet (G₄), the AC value was highest (5.37 ± 0.19), that reduced in G₅ (4.01 ± 0.15) and G₆ (3.71 ± 0.13) upon the administration of date fruit and date extract containing atherogenic diets, respectively.

Castelli's risk indexes are based on three major biomarkers of dyslipidemia. The current work indicated non-significant effect of treatment on CRI (I) and CRI (II) in normal rats. Nonetheless, atherogenic groups showed significant effect of treatments on these ratios. The obtained results for CRI (I) are 2.53 ± 0.08 , 2.39 ± 0.09 and 2.33 ± 0.07 in G₁, G₂ and G₃. Whereas, G₄, G₅ and G₆ rats exhibited 6.37 ± 0.20 , 5.01 ± 0.18 and 4.71 ± 0.17 , respectively. CRI (II) ranged from 0.96 ± 0.03 to 1.12 ± 0.04 in normal rats and 2.95 ± 0.10 to 4.40 ± 0.13 in the atherogenic ones.

Atherogenic ratios are potential assessors of CVD risks, the higher the ratio, the higher the risk of developing the cardiovascular ailments and vice versa (Ikewuchi and Ikewuchi, 2009). These lipid ratios are considered better predictors as compared to the single lipid markers owing to their association with a cluster of risk factors that may be unrelated to the cholesterol metabolism. For instance, the triglyceride to HDL ratio also negatively correlates with the insulin-stimulated glucose disposal (Brehm *et al.*, 2004). AIP is proposed as the predictive marker for CVD risk focusing on plasma atherogenicity based on the logarithm of triglyceride to HDL ratio (Upadhyay, 2015). It also correlates with the size of LDL and HDL particles and rate of fractional cholesterol esterification. Whereas, atherogenic coefficient is a measure of bad cholesterol (LDL, VLDL, IDL) with respect to good cholesterol (HDL). It reflects the atherogenic potential of entire spectrum of lipoproteins, hence, indicating the CVD risk (Nimmanapalli *et al.*, 2016). The Castelli's risk indexes (I & II) are based on three important lipid parameters i.e. TC, HDL and LDL that are the predictors of dyslipidemia associated cardiovascular events. The calculation of these ratios can be helpful in predicting the disease risk as well as the efficacy of therapeutic intervention (Bhardwaj *et al.*, 2013).

In the present work, it was apparent that all the atherogenic risk ratios in normal rats decreased with the provision of date fruit and extract-based diets. However, atherogenic diet elevated the ratios that were ameliorated significantly with date-based diets. Specifically, date extract proved to be more anti-atherogenic as compared to date fruit. In this regard, Dobiášová and Frohlich (2001) stated that high AIP value predicts higher risk for atherosclerosis or coronary artery disease. Likewise, Rosolova *et al.* (2014) found that the lipid modifying therapeutic interventions significantly reduces the AIP in

Table 3. Effect of diets on atherogenic risk ratios.

Diet	Experimental Group	Atherogenic Risk Ratio			
		AIP	AC	CRI (I)	CRI (II)
Normal Diet	G ₁	$0.30 \pm 0.01^{**}$	$1.53 \pm 0.04^{**}$	$2.53 \pm 0.08^{**}$	$1.12 \pm 0.04^{**}$
	G ₂	$0.28 \pm 0.01^*$	$1.39 \pm 0.06^*$	$2.39 \pm 0.09^*$	$1.01 \pm 0.04^*$
	G ₃	$0.27 \pm 0.01^*$	$1.33 \pm 0.06^*$	$2.33 \pm 0.07^*$	$0.96 \pm 0.03^*$
	F value	14.33**	32.15**	7.35*	10.34**
Atherogenic Diet	G ₄	$0.69 \pm 0.02^{**}$	$5.37 \pm 0.19^{**}$	$6.37 \pm 0.20^{**}$	$4.40 \pm 0.13^{**}$
	G ₅	$0.60 \pm 0.01^*$	$4.01 \pm 0.15^*$	$5.01 \pm 0.18^*$	$3.22 \pm 0.10^*$
	G ₆	$0.58 \pm 0.02^*$	$3.71 \pm 0.13^*$	$4.71 \pm 0.17^*$	$2.95 \pm 0.10^*$
	F value	52.89**	284.61**	36.68**	184.92**

Data are means \pm SD ($n = 10$); One-way ANOVA followed by Tukey's HSD multiple comparison tests; * = significant; ** = highly significant; NS = non-significant; AIP = \log TG/HDL; AC = (TC-HDL)/HDL; CRI (I) = TC/HDL; CRI (II) = LDL/HDL; G₁ = normal diet; G₂ = date fruit + normal diet; G₃ = date extract + normal diet; G₄ = atherogenic diet; G₅ = date fruit + atherogenic diet; G₆ = date extract + atherogenic diet.

atherogenic dyslipidemic patients from a high CV risk to low risk levels. The cholesterol containing diet increases the CRI (I) and CRI (II) ratio as well. Similar effect was observed by Fidèle *et al.* (2017) in a recent investigation. The researchers observed non-significant increase in the TC/HDL and LDL/HDL ratio in normocholesterolemic rats. However, the hypercholesterolemic rats treated with atorvastatin showed significant reduction in the ratios in a dose dependent manner.

The effect of dietary bioactives (thymoquinone and limonene) was assessed on the CVD risk ratios of atherogenic rats by Ahmad and Beg (2013). Their outcomes regarding the effect of bioactives on atherogenic rats corroborate with the present outcomes. The ratios increased in lipidemic groups as compared to the non-lipidemic ones. However, the treatments significantly restored the ratios. It is inferred that lowering the cholesterol and LDL content lowers these ratios, hence decreasing the perils of cardiovascular morbidity and mortality. Nevertheless, it was found through regression analysis that out of the four risk ratios, AIP contributes 30% to the CVD risk analysis followed by CRI (I) 20%, AC 16% and CRI II 13% (Bhardwaj *et al.*, 2013). Still, the ratios are helpful risk determinants and can give a quick risk analyses for further treatment and diet manipulation. In the present work, these ratios predicted that the rats fed with atherogenic diets are at high risk of developing cardiac complications as compared to the normal ones. Moreover, the date extract treatment proved to more effective in alleviating the CVD risk.

Serum oxidative stress indicators

Superoxide dismutase and catalase are the primary endogenous antioxidant enzymes in the line of defence against the toxic effects of oxygen radicals in the cells, and catalyse the dismutation of superoxide radical to H_2O_2 , thereby scavenging the free radicals. Means pertaining to the SOD level in normal rats showed lowest value in G_1 (16.15 ± 0.50 U/mL) that gradually improved in G_2 (17.28 ± 0.54 U/mL) and G_3 (17.04 ± 0.53 U/mL). Likewise, significant increase for this attribute was also reported in the atherogenic diet fed rats in which the SOD concentration was low in G_4 (8.62 ± 0.30 U/mL) but improved in date fruit fed group (G_5) 12.15 ± 0.43 U/mL, followed by date extract fed group (G_6) 11.97 ± 0.42 U/mL (Table 4).

Date-based diets intensified superoxide dismutase level throughout the study. In this context, normal rats presented 6.54 and 5.22% enhancement in SOD by date fruit and date extract containing diets, respectively. Similarly, in the atherogenic diet fed groups, 29.05 and 27.99% increase in SOD was

observed through the provision of date fruit and date extract supplemented diets, respectively.

Date-based diets imparted significant effect on serum catalase contents (CAT) of rats. In normal rats, mean catalase content for G_1 (91.47 ± 2.83 U/mL) was lower than that of G_2 (105.72 ± 3.27 U/mL) and G_3 (99.82 ± 3.09 U/mL) groups. Likewise, in atherogenic rats, G_4 group consuming control diet showed lesser catalase value of 41.24 ± 1.45 U/mL that increased to 53.28 ± 1.88 and 50.51 ± 1.78 U/mL in G_5 and G_6 groups taking date fruit and date extract containing functional diets, respectively (Table 4). The percent increase in serum catalase contents in G_2 was 13.48%, whilst in G_3 , date extract containing diet resulted in 8.37% increase as compared to control. Similarly, in the atherogenic groups, treatments G_5 and G_6 showed 22.60 and 18.35% increment.

Lipid peroxidation is a free radical and reactive oxygen species-mediated chain of reactions that when initiated leads to severe oxidative damage in serum and tissues. The lipid peroxides are unstable in nature and degrade rapidly into certain sub-products. Malondialdehyde (MDA) is the secondary product of lipid peroxidation and most popular marker of oxidative damage to cells (Grotto *et al.*, 2009). Serum MDA was considerably affected by the treatments in all groups (Table 4). Means regarding MDA (normal rats) yielded the highest value of 2.02 ± 0.06 nM/mL in G_1 that significantly reduced to 1.74 ± 0.05 and 1.53 ± 0.04 nM/mL in G_2 and G_3 groups, respectively. Similarly, in the atherogenic diet fed rats, highest MDA value was observed in G_4 (11.59 ± 0.40 nM/mL) that substantially decreased in G_5 (7.69 ± 0.27 nM/mL) and G_6 (8.26 ± 0.29 nM/mL). Date fruit have been found to possess more ameliorative properties as lowered 13.64 and 33.67% lipid peroxidation in normal and atherogenic rats, respectively. While, the rats fed with date extract-containing diets showed 12.54 and 28.76% reduction in normal and atherogenic rats, respectively.

Table 4. Effect of diets on serum oxidative stress indicators.

Diet	Experimental Group	Serum Oxidative Stress Markers		
		SOD (U/mL)	CAT (U/mL)	MDA (nM/mL)
Normal Diet	G_1	$16.15 \pm 0.50^*$	$91.47 \pm 2.83^*$	$2.02 \pm 0.06^{**}$
	G_2	$17.28 \pm 0.54^{**}$	$105.72 \pm 3.27^{**}$	$1.74 \pm 0.05^*$
	G_3	$17.04 \pm 0.53^*$	$99.82 \pm 3.09^*$	$1.53 \pm 0.04^*$
	F value	6.75^*	21.29^{**}	109.82^{**}

Table 4 (Cont.)

Atherogenic Diet	G ₄	8.62 ± 0.30*	41.24 ± 1.45*	11.59 ± 0.40**
	G ₅	12.15 ± 0.43**	53.28 ± 1.88**	7.69 ± 0.27*
	G ₆	11.97 ± 0.42*	50.51 ± 1.78*	8.26 ± 0.29*
	F value	245.57**	82.09**	66.62**

Data are means ± SD ($n = 10$); One-way ANOVA followed by Tukey's HSD multiple comparison tests; * = significant; ** = highly significant; NS = non-significant; G₁ = normal diet; G₂ = date fruit + normal diet; G₃ = date extract + normal diet; G₄ = atherogenic diet; G₅ = date fruit + atherogenic diet; G₆ = date extract + atherogenic diet.

Similar restorative effect of date fruit on endogenous enzymes and lipid peroxidation was observed by Ramadhas *et al.* (2014) who studied the effect of 200 mg/kg B.W. oral date fruit extract on lambda cyhaltrin induced toxic rats for 21 days. Date fruit treatment substantially improved the status of endogenous enzymes SOD and CAT in the toxic group by 35.68 and 32.88%, respectively. Lipid peroxidation significantly increased in the toxic group; however, date fruit extract treatment reduced the lipid peroxidation considerably. The reduction in serum lipid peroxidation through date fruit extract administration was also observed in another study conducted by Ragab *et al.* (2013). Ajwa date extract supplementation (300 mg/kg/d) to lead acetate induced stressed rabbits for 14 days decreased the MDA levels by 4.87%. The researchers also observed marked improvements in serum endogenous enzymes SOD and glutathione peroxidase. The results are also in harmony with the findings of Pushpa and Jayachitra (2015) who studied the effect of ethanolic date fruit extract (200 mg/kg) in normal and triton treated Wistar rats.

It is inferred that free radical scavenging potential of date fruit may perhaps be responsible for reducing the lipid peroxidation. Phytochemical moieties possess significant antioxidant potential i.e. capable of inhibiting the production of reactive oxygen species, thereby reducing the associated intracellular oxidative stress (Feng *et al.*, 2001). Moreover, the improvement in the endogenous antioxidant enzyme activity is the major approach towards reducing the exogenous stress inducing compounds.

Conclusion

Conclusively, the increased concentration of serum lipids and lipoprotein fractions fostered the development of atherosclerotic conditions. Atherogenic diet containing high amounts of fats and cholesterol contributed to the pathophysiological

conditions in various organs of the physiologic system, primarily due to the disturbed lipid moieties. From the current results, date fruit (Zahidi cultivar) was found effectual in restoring the endogenous enzyme status while the extract effectively improved the overall lipid profile. The present work thus clearly indicated that date fruit and its extract should be added in routine diet for improved health and disease prevention.

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