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Original article

Exploring the role of date pit based drink against hyperglycemia and hypercholesterolemia

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Summary. *Background:* The current study was used to probe the nutraceutical drinks containing Ajwa date pit powder against hyperglycemia and hypercholesterolemia. For this purpose, bio-evaluation studies were carried out on biochemical parameters in rodent modeling by using Ajwa roasted date pit drink. *Methods:* The Ajwa roasted date pit drink was developed under the aegis of a commercial automatic brewing machine and analyzed for sensorial and nutritional attributes. Moreover, effects of drinks on cholesterol and blood glucose levels in albino rats were explicated through efficacy studies. The collected data was subjected to statistical analysis using completely randomized design (CRD) through statistical through software Minitab 16. *Results:* Data reported that drink containing 5 and 10% Ajwa roasted date pit powder showed the maximum reduction in cholesterol, LDL and triglycerides 13.20, 15.23 and 9.13%, followed 8.01, 8.63 and 6.77%. Similarly, resultant drinks diminished the blood glucose level as 7.12 and 10.23% consecutively. These drinks also enhanced the insulin concentration as 7.56 and 3.25% in hyperglycemic rats. *Conclusion:* It is perceived that Ajwa date pit is endowed with vivid forthcoming to ameliorate the effect of oxidative stress, thus stopping the chain reactions implicated in the onset of chronic diseases. The outcomes of current project found Ajwa date pit most effective against obesity and allied discrepancies including hyperglycemia and hypercholesterolemia. Nutraceutical drinks have been proven significantly effective to reduce the glucose and cholesterol levels in experimental animals.

Key words: Ajwa date pit, efficacy study, hypercholesterolemia, hyperglycemia, coronary artery disease (CAD)

List of abbreviations

CRD	Completely Randomized Design
ANOVA	Analysis of Variance
LDL	Low Density Lipoprotein
CAD	Coronary Artery Disease
FH	Familial Hypercholesterolemia
FAO	Food and Agriculture Organization
T1DM	Type I Diabetes Mellitus
EDTA	Ethylenenediamine Tetra Acetic Acid
HDL	Density Lipoprotein
TBARS	Thio Barburic Acid Reactive Species
GSH	Glutathione
DNTB	Dithionitrobenzoic acid
AST	Aspartate amino Transferase
ALT	Alanine amino Transferase
ALP	Alkaline Phosphatase
DNPH	Di Nitro Phenyl Hydrazine
LCAT	Lecithin Acyl Transferase
TNF	Tumor Necrosis Factor

Background

Diabetes mellitus is a most prevalent disorder which is associated with higher sugar level that cause multifarious side effects (1). Familial hypercholesterolemia (FH) is an autosomal assertive genetic malady that engenders letups in low-density lipoprotein (LDL) cholesterol. It is an under apprehend and undertreated provocation of cardiovascular affliction (Turgeon et al., 2016). Approximately 80% of the people used plant based medicines for their primary healthcare worldwide (2). These plants based medicines waste products are promising source of an array of valued phytochemicals (3).

Dates are consumed in fresh or dried form; dried dates can be stored around the year for their consumption. Date pit is a byproduct of fruit which is comprised10-15% of total fruit weight and contains about 10% crude oil (4). The byproduct of date fruit is potential source of dietary fibres, protein, lipids, some vitamins, minerals and bioactive compounds (5). The growing demand of dates enhanced their production which reached 7.2 million tons in 2010 and approximately 720,000 tons of date–pits could be produced annually (i.e. considering 10% of the total fruit mass) (FAO, 2011).

Date pit comprises higher number of polyphenols mainly phenolic acids and flavonoids varied from 3102–4430mg GAE/100g fresh weight whereas antioxidant activity ranged from 580–929μmol of Trolox equivalents/g fresh weight. Due to higher nutritional profile, this byproduct can be used to prepare various functional food products (4, 6). Ajwa date extract possesses excellent antioxidant activity that is endorsed mainly to its rich contents of carotenoids, phenolics, melatonin and vitamins (7, 8, 9).

Date seeds polyphenols reduce liver serum malondialdehyde, serum lactate dehydrogenase and creatine kinase (10). These polyphenols also lower the blood cholesterol level in hypocholesterolemic rats due to antioxidant potential. It is conceivable that the antioxidants found in date seeds, especially phenolics and flavonoids, are the primary contributors to the ameliorating effect of date seeds on lipid peroxidation and ultimately reduction in the cholesterol level. They also improve glycemic control which may reduce glycosylated hemoglobin (HbA1c) levels (11, 12).

In type I diabetes mellitus (T1DM), they also enhance the secretion of insulin (13). Another dynamic mechanism of estimating the amount of insulin produced in the body, they proposed that C-peptide (connecting peptide), a 31-amino-acid polypeptide, represents the mid portion of the pro insulin molecule. During insulin secretion, it is enzymatically cleaved off and co-secreted in equimolar proportion with mature insulin molecules. Because synthetic insulin does not have such a peptide, the level of C-peptide can show how much insulin is being secreted in the body (14).

Materials and methods

The current research project was carried out in the Department of Food Science, Nutrition and Home Economics, Government College University Faisalabad. Materials used and protocols followed are described here in;

Procurement of raw materials

Ajwa date fruit, procured from local market of Faisalabad and then separated the date pits manually. For efficacy trial, male albino rats were procured from the Department of Pharmacology, Government College University Faisalabad. For biological assay, diagnostic kits were obtained from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia).

Preparation of raw materials

The separated pits were cleaned and separated by passing water over a metal strainer and then dried in hot air oven (Memmert UNE 200, Germany) at 50°C to remove moisture. Afterwards, the dried whole pits were roasted at 220°C for 15min in Home Coffee Roasting Machine (Model-SR500) and then cooled to ambient temperature. The roasted pits were grounded into dry powder (500um) using hammer mill (Fitz Mill model LH-DAS06). The resultant powder was stored in airtight food-grade container for further analysis.

Preparation of roasted date pit drink

The Ajwa roasted date pit drink was developed by using the method of (15) under the aegis of a com-

mercial automatic brewing machine (Model: Bunn, VP17-2).

Efficacy study

To evaluate the therapeutic potential of developed functional drinks against life style related disorders such as hyperglycemia and hypercholesterolemia, a bioefficacy trial was conducted. For the purpose, 120 male albino rats were housed in the Animal Room of Institute of Food Science, Nutrition and Home Economics, Government college university, Faisalabad. The rats were acclimatized by feeding basal diet for a period of one week. The environmental conditions as temperature (23±2 °C) and humidity (55±5%) were maintained throughout the study duration with 12 hr light-dark period. At the initiation of study, some rats were sacrificed to establish a baseline trend. During efficacy trial, four independent studies were carried out involving normal, hyperglycemic and hypercholesterolemic rats.

Study I: Normal rat group (control group), the control rats were fed with the normal diet comprised 10% corn oil, 10% protein, 66% starch, 10% cellulose, 3% mineral and 1% vitamin mixture.

Study II: Hypercholesterolemic rat group, the hypercholesterolemic rats were prepared by feeding a normal diet containing 1.5% cholesterol and 0.5% cholic acid.

Study III: Diabetic rat group, the diabetic rats were induced by providing high sucrose diet (40%).

Study IV: Diet containing cholesterol and sucrose in amount of 1% and 40% respectively.

The study was comprised of rats (120), divided in four equal groups (30). In group I, rats were fed on normal diet whilst, in group II hypercholesterolemia was induced through higher cholesterol diets, group III (hyperglycemia), high sucrose diet was given to the rats. Moreover, for group IV, combination of high cholesterol and high sucrose diets were given. The resultant functional drinks were given to the respective groups. During 8 weeks of efficacy trial, administration of date pit based functional drinks was assured to evaluate their therapeutic effects. Physical parameters like feed & drink intakes and body weight were also determined. At the end of study, the overnight fasted rats were decapitated (by smelling chloroform) and blood was collected in ethylenenediamine tetra acetic acid **(**EDTA) coated tubes. Initially, blood samples were tested for different hematological characteristics like red & white blood cells indices. Later, the collected samples were subjected to centrifugation for serum separation. The respective sera from each group were analyzed for different biomarkers by Microlab 300 (Merck, Germany). The respective commercial kits were used for the estimation of various biochemical parameters like total cholesterol, LDL, HDL, triglycerides, glucose and insulin levels. Additionally, liver and kidney soundness tests were also performed to estimate the safety & renal modulating perspectives of functional drinks (Table 1).

Feed and drink intakes

The net feed intake was determined on daily basis by excluding spilled diet from the total diet. Similarly, intake of resultant drinks of each rat was also measured daily by monitoring the differences in the graduated bottles (16).

Body weight gain

Weight gain of experimental rats was determined on weekly basis throughout the study period to evaluate any suppressing effect of functional drinks on the body weight.

Table 1. Diet plan for efficacy studies

	(Study I) Normal diet			**(Study II) High cholesterol diet**			**(Study III) High sucrose diet**			**(Study IV) High cholesterol + high sucrose diet**		
Groups	1	2	3	1	2	3	1	2	3	1	2	3
Feed plan	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2	T_0	T_1	T_2

T_0: placebo; T_1: Drink containing 5% Ajwa roasted date pit powder; T_2: Drink containing 10% Ajwa roasted date pit powder

Serum separation

In commercially available red topped tubes, blood samples were collected and then were allowed to clot at room temperature for 30 min. Further, clotted part was removed after centrifugation through Centrifugal Machine (Model: 800, China) @ 4000 rpm for 6 min to obtain serum (17).

Serum lipid profile

Serum cholesterol level was determined by using CHOD–PAP method through the guidelines of (18). Likewise, high density lipoprotein (HDL) was estimated by Cholesterol Precipitant method as elaborated by (19). Furthermore, low density lipoprotein (LDL) and triglycerides level was calculated through liquid triglycerides (GPO–PAP) method following the guidelines of (18).

Serum glucose and insulin levels

Hypoglycemic response of date pit based functional drinks was determined by measuring the serum glucose and insulin levels of experimental rats. In each study, glucose concentration of rats was determined by GOD-PAP method following the protocol of (20). Similarly, insulin level was calculated by adopting the guidelines of (21).

Antioxidant status

The concentration of glutathione content was investigated by using the method of (22). The colored product of GSH + DTNB in the protein free supernatant was recorded at 412 nm and calculated as nmol/mg protein. Likewise, thiobarburic acid reactive species (TBARS) was also determined through the instructions of (23).

Liver and kidney functioning tests

Liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were performed. The levels of AST and ALT were assessed by the dinitrophenylhydrazene (DNPH) method using Sigma Kits 59-50 and 58-50, respectively whilst ALP by Alkaline Phosphates–DGKC method (24). Bilirubin total was determined by Jendrassik-Grof method (25). Moreover, the urea and creatinine concentrations were also recorded by GLDH and Jaffe-method, respectively using commercial kits (26, 27) to examine the renal functionality of tested rat groups.

Statistical analysis

The collected data was subjected to statistical analysis using completely randomized design (CRD) through statistical software Minitab 16. Moreover, analysis of variance (ANOVA) was used to determine the level of significance (28).

3-Results and Discussion

Physical parameters

Drink, feed, and body weight

The mean values for drink intake in study I during 1st week was varied from 15.94±0.75 to 22.46±1.10, 15.93±0.67 to 22.45±0.99 and 15.94±0.66 to 22.46±1.08mL/rat/day for T_0, T_1 and T_2, respectively at the end of study. Similarly, in study II, drink intake in T_0, T_1 and T_2 were 18.05±0.89, 17.97±0.90 and 17.93±0.87mL/rat/day that increased to 24.57±1.10, 24.12±0.99 and 24.32±1.12 mL/rat/day, correspondingly at the end of study. Likewise, in study III, mean values were ranged from 18.38±0.89 to 24.81±1.01mL/rat/day in T_0, T_1 and T_2 groups at the termination of study. Moreover, mean values for increasing trend in study IV were 25.53±1.24, 25.09±1.12 and 25.16±1.21mL/rat/day in T_0, T_1 and T_2 groups, respectively. Maximum feed intake (study I) varied from T_0 (17.09±0.0.78 g/rat/day) to T_1 (16.96±0.96) at the initiation and termination of period.

Similarly, in study II and III, mean values were recorded as 16.39±0.76 & 21.4±1.11, 16.25±0.88 & 21.6±0.98 and 16.33±0.72 & 21.64±0.93g/rat/day in T_0, T_1 and T_2 groups at 1st and 8th weeks, respectively. Likewise, increased values were reported as 22.76±1.22, 22.36±0.98 and 21.75±1.10g/rat/day in T_0, T_1 and T_2 (study III) in trial periods. In study IV, overall mean values for feed intake were 14.03±0.65, 13.89±0.56 and 13.91±0.42 g/rat/day in T_0, T_1 and T_2, respectively that subsequently increased to 19.61±0.81, 19.37±0.76 and 19.01±0.62 at 8th week (Figure 1, 2). It is evident from the figure 3 that in study II, T_1 and T_2 groups caused re-

duction as 8.16 and10.2%, respectively whereas in study, percentage reduction values were 4.98 & 6.79%, respectively in study III. Similarly, study IV, T_2 caused 6.71% trailed by T_1 as 4.91% as compared to control.

The current results are in line with findings of researchers including (29, 30), they determined the non-significant effect of fluid intake containing date palm nutraceutical components. In all studies, there was a progressive increase in feed intake with passage of time however, the differences were more pronounced in placebo group. Similarly, (29) noticed non-significant less feed consumption in rats administrated on

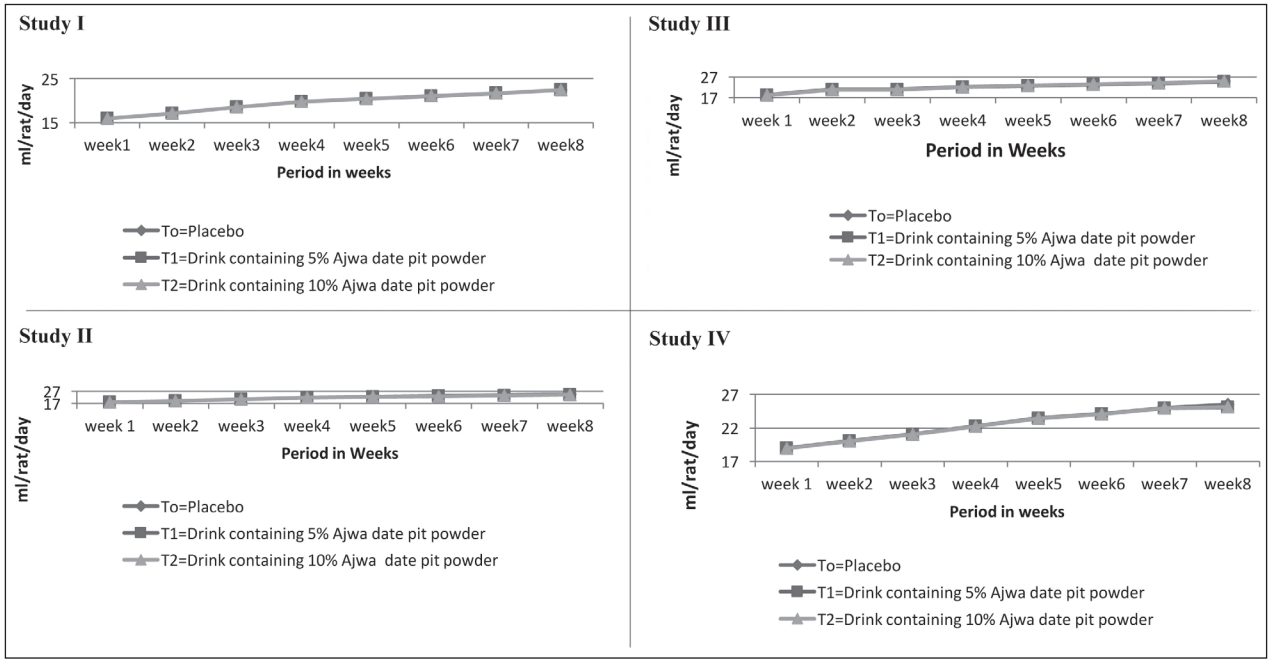

Figure 1. Drink intake in study I, II, III and IV (ml/rat/day)

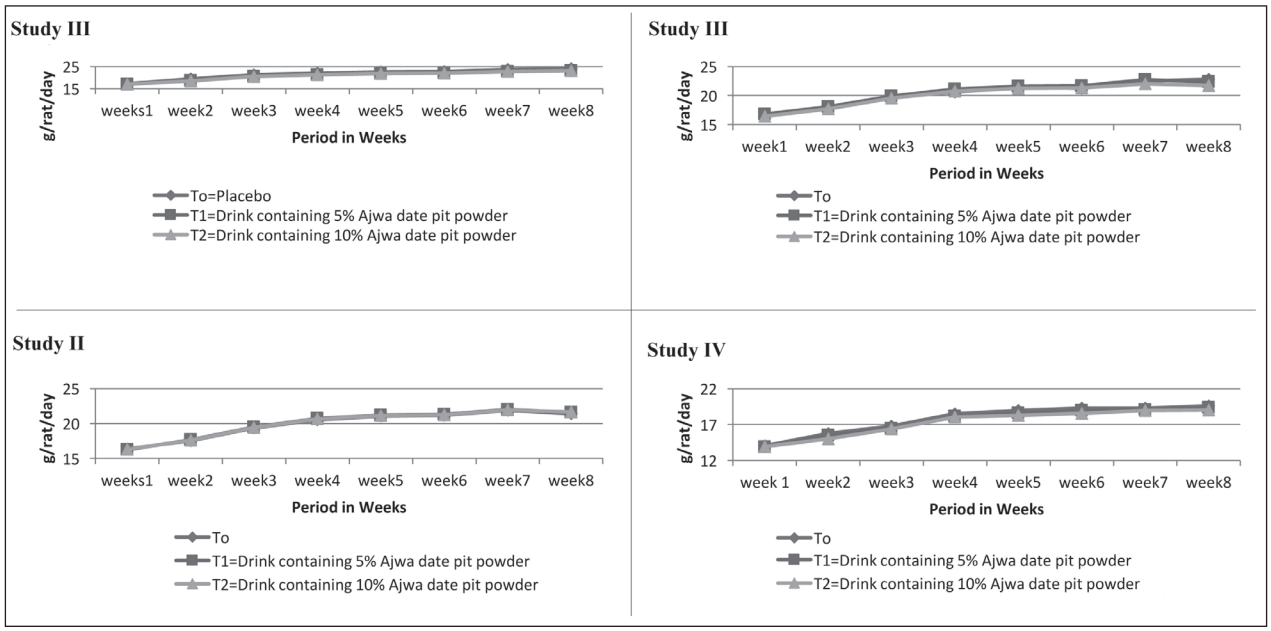

Figure 2. Feed intake in study I, II, III and IV (g/rat/day)

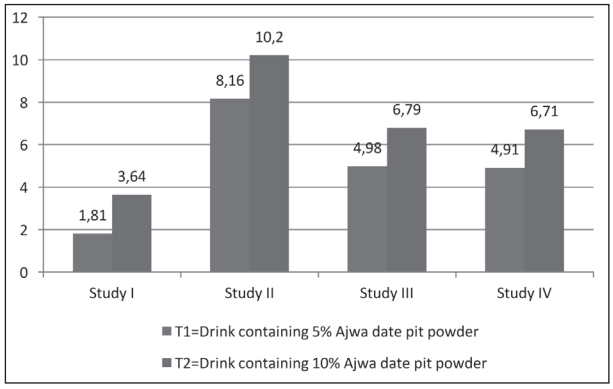

Figure 3. Percent reduction in body weight at 8th week as compared to control

date based polyphenol extract. They were of the view that improved satiety, feed efficiency and suppressing appetite may be the possible routes.

The anti-obesity effect of date palm was probed by (31); the findings from the studies made it clear that the rats which were given high fat diet in combination with 100mg/kg body weight of *Phoenix dactylifera* extract gained 36% less weight when compared with the control group fed on high fat diet. They further narrated that bioactive components like antioxidants, phenolics and flavonoids in *Phoenix dactylifera* may stimulate hepatic micrsomal cytochrome p450 dependent aryl hydrolase activity which is believed to be involved in the hydroxylation of endogenous steroids such as cholesterol and there by increases the catabolic conservation of cholesterol to bile acids in liver. Moreover, they suggested that sterol in *Phoenix dactylifera* reduces the absorption of cholesterol and thus increasers the fecal excretion of steroids that results in decrease of body lipids. However, flavonids from *Phoenix dactylifera* may augment the activity of lecithin acyl transferase (LCAT), which regulates blood lipids. LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later, in liver cells. Several studies have showed that increase in HDL-C is associated with decrease in CAD (32, 33).

Lipid profile

Means values for cholesterol in study I (Table 2) showed that reported values of cholesterol in T_0 (78.25**±4.01mg/dL) that significantly reduced to** 75.91**±3.72mg/dL (**T_1) and 75.03**±3.24mg/dL** (T2), respectively. Similarly, in hypercholesterolemic rats (study II), recorded values for T_0, T_1, T_2 were 130**±7.42,** 120.17**±4.09, and** 114.36**±5.95 mg/dL,** respectively, mg/dL and for. Likewise, in diabetic rats, (study III) cholesterol level was reduced from T0 (96**±2.95mg/dL)** to (87.82**±3.08 mg/dL)** T_2. Moreover, in study IV,

Table 2. Effect of Ajwa date pit drinks on cholesterol, LDL, HDL, and Triglycerides

	LDL (mg/dL) Treatments			Cholesterol (mg/dL) Treatments		
Studies	**T_o**	**T_1**	**T_2**	**T_o**	**T_1**	**T_2**
Study I	28.16±**0.68**^{a}	27.28±**0.65**^{b}	26.69±**0.61**^{c}	78.25±**4.01**^{a}	75.91±**3.72**^{b}	75.03±**3.24**^{b}
Study II	59.63±**1.09**^{a}	55.07±**1.71**^{b}	51.13±**1.59**^{c}	130±**7.42**^{a}	120.17±**4.09**^{b}	114.36±**5.95**^{c}
Study III	45.12±**1.18**^{a}	42.85±**1.13**^{b}	41.01±**1.08**^{c}	96±**2.95**^{a}	90.58±**2.89**^{b}	87.82±**3.08**^{c}
Study IV	71.23±**2.33**^{a}	65.08±**2.31**^{b}	60.38±**2.28**^{c}	157.12±**6.09**^{a}	144.53±**7.96**^{b}	136.38±**7.65**^{c}
	T_o	**T_1**	**T_2**	**T_o**	**T_1**	**T_2**
Studies	**HDL (mg/dL)**			**Triglycerides (mg/dL)**		
Study I	36.65±**1.09**^{b}	37.12±**1.08**^{a}	37.43±**1.75**^{a}	69.12±**1.89**^{a}	67.07±**1.08**^{b}	67.04±**1.96**^{b}
Study II	29.63±**1.21**^{c}	30.55±**1.24**^{b}	31.59±**1.29**^{a}	98.32±**2.46**^{a}	92.51±**3.38**^{b}	92.06±**3.45**^{c}
Study III	32.12±**1.38**^{b}	32.69±**1.35**^{b}	33.52±**1.49**^{a}	75.63±**2.09**^{a}	72.60±**2.67**^{b}	71.83±**2.89**^{c}
Study IV	26.93±**1.05**^{c}	27.50±**1.15**^{b}	28.86±**1.19**^{a}	108.16±**5.02**^{a}	100.84±**5.43**^{b}	98.28±**5.68**^{c}

cholesterol values were observed in T_0 (157.12**±6.09 mg/dL) that significantly reduced in** T_2 (136.38**±7.65 mg/dL) followed by** T_1 (144.53**±7.96 mg/dL).** Means regarding LDL (Table 3) indicated maximum value 28.16**±0.68mg/dL in** T_0 substantially reduced to 27.28**±0.65 and** 26.69**±0.61mg/dL in** T_1 and T_2 groups, respectively in study I. A similar trend was observed in study II, T_0 exhibited the highest LDL 59.63**±1.09 mg/dL while reduced in** T_1 55.07**±1.71 and** T_2 51.13**±1.59mg/dL. In study III, results were obtained in the same order as** 45.12**±1.18**mg/dL, 42.85**±1.13**mg/dL and 41.01**±1.08**mg/dL for T_0, T_1 & T_2 treatments. Study IV resulted in high LDL in T_0 i.e. 71.23**±2.33**mg/dL however, the values for T_1 and T_2 consequentially decreased to 65.08**±2.31** and 60.38**±2.28**mg/dL, in that order. Mean values regarding HDL (Table 2) in study I for T_0, T_1 and T_2 groups were 36.65**±1.09,** 37.12**±1.08 and** 37.43**±1.75mg/dL.** Similarly, HDL values were 29.63**±1.21,** 30.55**±1.24 and** 31.59**±1.29mg/dL for** T_0, T_1 and T_2, correspondingly (study II). Mean value for T_0 in study III was 32.12**±1.38mg/dL that increased non-significantly to** 32.69**±1.35 and significantly to** 33.52**±1.49mg/dL in** T_1 and T_2 groups, respectively. Likewise, in study IV, HDL values for T_0, T_1 and T_2 were 26.93**±1.05,** 27.50**±1.15 and** 28.86**±1.1949mg/dL, respectively.** The mean values for triglycerides (Table 2) in study I were 69.12**±1.89**, 67.07**±1.08** and 67.04**±1.96**mg/dL, for T_0, T_1 and T_2 groups. Similarly, in study II, mean values for T_0, and T_2 were varied from 98.32**±2.46** to 92.06**±3.45**mg/dL, respectively. However, triglycerides level was increased to 75.63**±2.09**mg/dL in T_0 group but (T_1) and (T_2) suppressed the values for this trait to 72.60**±2.67** and 71.83**±2.89**mg/dL, respectively (study III). Likewise, in study IV, triglyceride levels were reduced for T_1 and T_2 groups i.e. 100.84**±5.43** and 98.28**±5.68**mg/dL, respectively as compared to T_0 i.e. 108.16**±5.02**mg/dL (Table 2).

Similarly, data reported from the findings of (30), they explicated that diet containing defatted date seed flour at 1.5, 2.5 and 5.2 % concentration significantly lowered the LDL, plasma triglycerides and total cholesterol in rats.

The current results supported the findings of (34), they determined that bread formulated with date pit powder (10% and 15%) caused a significant reduction of LDL as 38.96% and 48.29% in hypercholesterolemic albino rats.

The present findings are in harmony with the results documented by (10) who suggested that date pit bioactive antioxidants possess a protective effect against in vivo oxidation damage, they fed Wistar rats

Table 3. Effect of Ajwa date pit drinks on glucose (mg/dL), insulin (µU/mL), glutathione (mg/L), and TBARS (µmol/L)

	Glucose (mg/dL)			**Insulin (µU/mL)**		
	Treatments			**Treatments**		
Studies	**T_o**	**T_1**	**T_2**	**T_o**	**T_1**	**T_2**
Study I	81.23**±3.08**^{a}	79.24**±3.59**^{b}	78.64**±3.45**^{c}	9.12**±0.73**^{c}	9.23**±0.57**^{b}	9.30**±0.65**^{a}
Study II	101.25**±5.66**^{a}	96.88**±4.98**^{b}	94.04**±4.25**^{c}	7.12**±0.64**^{c}	7.29**±0.52**^{b}	7.42**±0.42**^{a}
Study III	140.12**±6.25**^{a}	130.14**±6.28**^{b}	125.79**±6.48**^{c}	6.63**±0.55**^{c}	6.85**±0.56**^{b}	7.13**±0.48**^{a}
Study IV	136.14**±6.48**^{a}	127.66**±5.79**^{b}	123.60**±5.02**^{c}	5.59**±0.43**^{b}	5.71**±0.35**^{b}	5.96**±0.40**^{a}
	T_o	**T_1**	**T_2**	**T_o**	**T_1**	**T**
Studies	**Glutathione (mg/L)**			**TBARS (µmol/L)**		
Study I	50.12±2.3	51.95±2.45	52.70±2.75	6.98±0.32	6.75±0.36^b	6.63±0.34^c
Study II	38.65±1.52	43.03±2.12	44.12±2.31	10.23±0.52	9.21±0.42^b	8.97±0.38^c
Study III	40.63±1.96	44.34±1.75	44.82±1.26	8.12±0.41	7.45±0.39^b	7.36±0.38^c
Study IV	43.12±2.14	49.21±2.22	50.16±2.56	11.26±1.22	991±0.43^b	9.66±0.47^b

with a basal diet containing 0, 70 and 140g/kg date pits for 30 days and observed significant decrease in the serum cholesterol of the experimental subjects. In a recent study, (34) fed male experimental rats with bread fortified with date pits at the rate of 10% and 15%, the data gathered through biochemical analysis of experimental animals revealed 1.84% and 7.95 % decrease in the serum cholesterol level, correspondingly. The research of (29) is in accordance with the instant findings of present study they fed male albino rats with date palm extract for 30 days at the rate of 10mg/100g diet, 20mg/100g diet, 30mg/100g diet and 40mg/100g diet and recorded progressive decrease in the blood cholesterol level as 2.76%, 10.57%, 13.28% and 17.08%, respectively. The findings of Al-Saif et al. (2007) supported the present investigation of cholesterol reduction by date palm phytochemicals, they fed male hamsters by incorporating date pulp in diet at the rate of 50% and observed 11.03% reduction in blood cholesterol when compared with the control group.

Gastrointestinal lipid digestion and absorption requires the enzymatic hydrolysis of triglycerides; thus, the inhibition of pancreatic lipase is a possible strategy to prevent hyperlipidemia by decreasing lipid absorption. Polyphenols have been reported to decrease pancreatic lipase activity (35, 36); however, the direct interaction with the pancreatic lipase active site is not the only mechanism of inhibition. Triglycerides must emulsify into fat droplets to generate a suitable substrate for pancreatic lipase activity (37).

The results are in line with the instant findings of (34) who formulated pan bread by supplementing with date pit powder (10% and 15%) and fed to the albino rats. They observed the momentous reduction 38.96% and 48.29% in blood LDL level, improvement in HDL as 13.12 and 37.56%, and reduction in in triglycerides as 7.88% and 24.74%. Likewise, (10) documented similar findings by carrying out investigation to explore LDL lowering ability of date pits, by feeding experimental rats with date pit powder at the rate of 7% and 14% and recorded substantial reduction 9% and 36% in LDL level, respectively. Similarly, (30) confirmed that diets supplemented at the rate of 1.5% and 2.5% with date pit powder caused reduction in LDL level as 52.18% and 67.22% and enhancement in HDL as 15.25% and 62.23%. They also observed the reduction in serum triglyceride as 6.12% for rats fed with diet supplemented with date pit fiber (2.5%) and (17.65%) for diet supplemented 5.2% with date pit fiber. Likewise, the data of present investigation is also confirmed by the studies carried out by (10); they recorded momentous decrease of 5.4% and 7.49% in serum triglyceride levels of albino rats by administering 7% and 14% date pit powder supplemented diet, respectively.

Glucose

It is depicted in study III exhibited highest reduction as 7.12 and 10.23 % followed by study IV, 6.23 and 9.21%, study II 4.32 and 7.12% and study I 2.45 and 3.19% in T_1 and T_2 groups, respectively (Figure 4). Ajwa date pit based functional drinks enhanced the concentration of enzyme glucose 6- phosphate dehydrogenase through enhancing the secretion of insulin that increases the influxes of glucose into pentose monophosphate shunt by lowering high blood glucose levels (38, 39; 40).

Halaby et al. (2014) determined that male albino rats fed with 10 and 15% date pit powder fortified bread and observed reduction in serum glucose level as 21.46 and 30.79% whilst exhibited significant enhancement in insulin level as (48.17% & 50.29%). Likewise, (34); recorded 1.04% decrease in serum glucose level by feeding rats with diet containing 7% date pit powder. Date pit polyphenols caused reduction in serum glucose by inhibiting α-Amylase and α-glucosidase activity. These enzymes are involved in starch breakdown and intestinal absorption, respectively. The first enzyme is involved in the digestion of carbohydrates to produce simpler saccharides, whereas, the second is involved in their absorption. It is believed

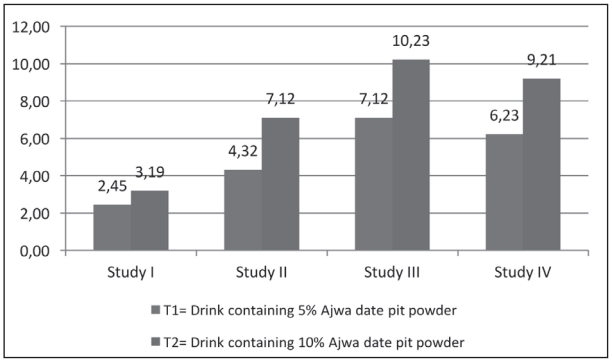

Figure 4. Percent reduction in glucose as compared to control

that inhibition of the two enzymes would result in a lower blood glucose levels after a rich carbohydrate diet. They documented 6.3, 7.9 and 24.2% decrease in serum glucose by administering 33, 50 and 60 g/l date extract in rodent modeling (41).

Insulin

It is depicted from the figure 5 that in study I and II, the treatments T_2 exhibited momentous increase in insulin level as 2.01 & 4.25% followed by T_1 as 1.23 & 2.33%, respectively whereas, in study III and IV, T_2 and T_1 showed momentous enhancement of insulin level as 7.56 & 6.69% and 3.25 & 2.21%, respectively.

Likewise, (13) successfully tested the efficacy of date pit extract on the glycemic control of type I diabetes mellitus (T1DM) in rats. This glycemic control was endorsed to increase in endogenous insulin secretion triggered by date pit polyphenols. (14) discussed the mechanism of estimating the amount of insulin produced in the body, they proposed that C-peptide (connecting peptide), a 31-amino-acid polypeptide, represents the mid portion of the pro insulin molecule. During insulin secretion, it is enzymatically cleaved off and co-secreted in equimolar proportion with mature insulin molecules. Because synthetic insulin does not have such a peptide, the level of C-peptide can show how much insulin is being secreted in the body. The studies of (13) showed significantly high C-peptide values among rat group fed for the period of 8 weeks with 10ml date pit extract daily, compared with control rat group. The mechanism underlying in increased insulin sensitivity with date pit drink is endorsed to the anti-oxidative effect of date pit polyphenols, as they improve the number of insulin receptor binding sites

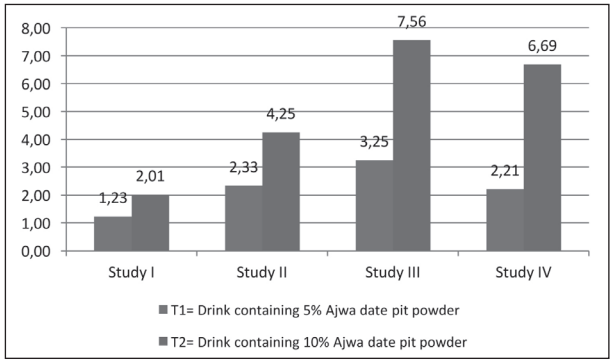

Figure 5. Percent increase in insulin as compared to control

leading to higher glucose uptake. The capability of Ajwa date pit drink polyphenols to suppress the blood glucose and insulin level following carbohydrate ingestion in rats, might be due to its ability to inhibit the activities of α-amylase & α-glucosidase in the intestine that balance the glucose and insulin level, enhance the insulin binding to the adipocytes and promote the intracellular glucose transporter in the myocytes. (41).

Antioxidant status

Glutathione

Mean values for glutathione contents in T_0 (50.12±2.3 & 38.65±1.52, 51.95±2.45 & 43.03±2.12mg/L, and T_2 (52.70±2.75 & 44.12±2.31mg/L) in T_0, T_1 and T_2 groups, (study I & II). In study III, level for glutathione was 40.63±1.96mg/L in T_0 that elevated in T_1 (44.34±1.75mg/L) and T_2 (44.82±1.26mg/L) groups. In study IV, glutathione content was suppressed (43.12±2.14mg/L) in control group whereas, high values 49.21±2.22and 50.16±2.56mg/L were observed in T_1 and T_2 groups, respectively (Table 3).

During stress condition, cellular respiration produces hydrogen peroxide that triggers the cascade of deleterious reactions. The antioxidants like glutathione halts this process by converting the hydrogen peroxide into water thus helps body to regain its normal oxidation potential (42, 43, 44) they administered date pit coffees orally at different concentrations (50, 100 and 150 mg/kg body weight/day) and observed 3.61, 5.08 and 4.56% reduction in glutathione level, respectively. Likewise, (10) documented 1.15 and 1.13% decrease in glutathione level in rats fed with 7 and 14% date pit powder, respectively.

Thiobarbituric acid reactive substances (TBARS)

Highest TBARS value noted for T_0 (10.23±0.52**µmol/L) in study II was significantly reduced in T_1** (9.21±0.42**µmol/L) and T_2** (8.97±0.38**µmol/L) groups.**

In study III, results were obtained in the same order as 8.12±0.41**µmol/L,** 7.45±0.39 and 7.36±0.38**µmol/L for T_0**, T_1 & T_2 treatments. Similar diminishing trend in groups consuming the date pit

drinks was recorded for TBARS in study IV and value for T_0 was 11.26±1.22**µmol/L that reduced substantially to** 9.91±0.43 and 9.66±0.47**µmol/L in T**$_1$ and T_2 groups, respectively. In this context, (45) elucidated that the LDL-associated aldehydes (TBARS) levels were significantly reduced by date extract and the date seed extract by upto 47% and 83%, respectively.

In this context (46) observed date pit polyphenols exhibit soothing action on elevated TBARS by hindering the production of superoxide and chelating metal ions; they narrated 48.78% decreasing trend in serum MDA level of normal and lead acetate induced oxidative stress rats after consuming 300 mg/kg body weight of Ajwa date extract. (47) they treated the experimental animals with Degla date extract (4 mL/kg b.w, corresponding 3.34 mg GAE/Kg b.w) in addition to low (0.5 g/L) and high (2 g/L) dose level of DCA and observed significant dose dependent reduction (18.18 & 16.16%) in MDA level, respectively. Earlier, the antioxidant potential of Deglet Nour date extract against dimethoate induced-oxidative stress in rat liver was studied by (48), administering 4ml/kg of body weight for two months and observed 22.72% decrease in MDA level.

Kidney functioning tests

Means values for urea (Table 10) in T_0, T_1 and T_2 groups were 20.32**±5.08**, 19.89**±5.18** and 19.68**±4.89** mg/dL, respectively, in study I. In study II, there was noted high urea level 31.12**±1.64** mg/dL in T_0 group that reduced to 30.15**±2.02** mg/dL in T_1 and 29.36**±1.09** mg/dL in T_2 group. Similarly, in study III, rats showed uplifted urea level (26.34**±1.51**mg/dL) in T0 group whereas, its level reduced to 25.47**±1.29** and 24.76**±1.08** mg/dL in T_1 and T_2 groups, respectively. Maximum urea was in T_0 group (35.21**±2.23** mg/dL) followed by T_1 (33.49**±1.98** mg/dL) and T_2 (33.02**±2.90** mg/dL) in study IV. In study I, mean values for creatinine were 0.79**±0.056**, 0.78**±0.098** and 0.77**±0.034** mg/dL for T_0, T_1 and T_2 groups, respectively. Likewise, in study II, means for creatinine in T_0 was 0.96**±0.065** mg/dL trailed by significant reduction in T_1 (0.94**±0.072** mg/dL) and T_2 (0.92**±0.054** mg/dL). In study III comprising of high sucrose diet, T_0 showed highest creatinine level (1.04**±0.056** mg/dL) that momentously decreased to 1.01**±0.037** and 1.00**±0.025** mg/dL in T_1 and T_2 groups, respectively. Considering the results of study IV, maximum creatinine 1.65**±0.054**mg/dL was recorded in T_0 group (Placebo) that significantly reduced to 1.55**±0.091** mg/dL in T_1 (drink containing 5% Ajwa roasted date pit powder) and 1.37**±0.054**mg/dL in T_2 (drink containing 10% Ajwa roasted date pit powder) groups. Data reported from (47) they treated the experimental animals with Degla date extract (4 mL/kg b.w, corresponding 3.34 mg GAE/Kg b.w) in addition to low (0.5 g/L) and high (2 g/L) dose level of DCA and observed significant dose dependent decrease in urea (18.57 & 23.07%), uric acid (16.67 & 21.07), and creatnine level (33.33 & 25%). Earlier, Ragab et al. (2013) narrated 14.55% decreasing trend in serum urea and 19.78% creatinine level of normal and lead acetate induced oxidative stress rats after consuming 300 mg/kg body weight of Ajwa date extract. Likewise, according to (34) date pit powder is effective against hyperglycemia, they fed male albino rats with 10 and 15% date pit powder fortified bread and narrated decrease in serum urea level as 28.90 and 33.61%, and creatinine level as 34.86 and 46.78% respectively, furthermore they recorded 33.73 & 35.36% reduction in uric acid level. Similarly, (29) investigated that male albino rats fed with date palm extract for 30 days at the rate of 10, 20, 30 and 40mg/100g diet and recorded progressive decrease in the blood urea level as 2.16, 4.92, 9.2 and 13.27 % as well as also decreasing trend in creatinine level as 1.96, 6.53, 11.11 and 15.68 %, respectively.

Liver function tests

Although, in study II higher ALT value (53.88±2.35IU/L) was noted in T_0 group consuming control drink that reduced in T_1 (44.13±1.87IU/L) and T_2 (42.89±2.12IU/L) groups taking roasted date pit drinks. Besides, in study III, mean for ALT in T_0 was 49.65±1.89 IU/L that decreased to 44.13±1.54IU/L in T_1 and 44.7±1.98IU/L in T_2. In study IV, mean for T_0 was 55.84±2.11IU/L while, T_1 and T_2 groups provided roasted date pit drink showed significant reduction in ALT level i.e. 48.11±2.22 and 46.63±2.35IU/L, respectively (Table 4).

Means pertaining to AST level in study II, showed high value in T_0 (119.52±4.99IU/L) as compared to T_1

(112.45±3.22IU/L) and T_2 (109.08±2.79IU/L). High sucrose diet (study III) given to rats resulted in elevated AST level in T_0 (92.33±4.55IU/L) group whilst its level was comparatively low in T_1 (87.94±2.56IU/L) and T_2 (85.77±3.13IU/L) groups consuming Ajwa date pit drinks. Likewise, in study IV, AST value for T_0 was 129.79±5.64IU/L trailed by T_1 and T_2 groups having mean values 116.79±3.98and 109.62±3.51IU/L, respectively for this trait (Table 4).

Mean values for ALP were reduced to T_1 (234.75±5.87 IU/L) and T_2 (220.9±8.26 IU/L) as compared to T_0 (261.61±10.45IU/L), respectively. Likewise, in study III, higher ALP value was recorded in T_0 (250.46±11.65IU/L) as compared to T_1 (223.63±7.56IU/L) and T_2 (219.91±8.21IU/L) groups. Mean ALP value in study IV for T_0 group was 302.09±12.54 trailed by T_1 (283.09±11.54IU/L) and T_2 (272.88±10.65IU/L) groups (Table 4). Mean values of bilirubin study II, T0 (1.35±0.08mg/dL) reduced to T_1 (1.22±0.06mg/dL) and T_2 (1.18±0.02mg/dL) groups whilst 1.16±0.02, 1.05±0.05mg/dL and 1.01±0.01mg/dL were recorded for T_0, T_1 & T_2, respectively. In study IV, mean for bilirubin in T_0 was 1.40±0.09mg/dL, whereas, T_1 and T_2 groups showed significant reduction i.e. 1.30±0.07and 1.23±0.04mg/dL, respectively (Table).

Studies on male wistar rats conducted by (10) proved that date pits reduce ALT level owing to the presence of high amounts of antioxidants. They fed male Wistar rats with a basal diet containing 70g/kg date pits for 30 days, it was observed that date pits significantly reduced blood ALT level by 13.81%. Similarly, (9) studied the protective role of ajwa date against the hepatotoxicity induced by ochratoxin and observed 15.92% reduction in ALT level at a dose of 1g/kg body weight. Likewise, (13) observed 0.44% reduction in ALT level by giving daily ingestions of 10 ml of date pit extract for 8 weeks. Likewise, (34) fed albino rats on bread fortified 10 and 15% with date pits they noticed 54.21 & 62.18% reduction in ALT. Earlier, the antioxidant potential of Deglet Nour date extract against dimethoate induced-oxidative stress in rat liver was studied by (48), administering 4ml/kg of body weight for two months and observed 33.33% decrease in ALT serum level. Earlier, (49) observed 38.26% reduction in blood ALT level in thioacetamide-Induced hepatotoxicity in rats administered with 4ml/kg of body weight.

In fact, (50) proved the antioxidant and the anti-mutagenic activity of the aqueous date extract, as monitored by the inhibition of lipid per-oxidation and protein oxidation and also by the aptitude to scavenge

Table 4. Effect of Ajwa date pit drinks on serum ALT (IU/L), AST (IU/L), and ALP (IU/L)

	Serum ALT (IU/L)			Serum AST (IU/L)		
	Treatments			Treatments		
Studies	**T_0**	**T_1**	**T_2**	**T_0**	**T_1**	**T_2**
Study I	44.41±1.56	43.6±1.52	43.04±1.24	68.89±2.56	65.52±2.89	64.69±3.01
Study II	53.88±2.35	44.13±1.87	42.89±2.12	119.52±4.99	112.45±3.22	109.08±2.79
Study III	49.65±1.89	44.13±1.54	44.7±1.98	92.33±4.55	87.94±2.56	85.77±3.13
Study IV	55.84±2.11	48.11±2.22	46.63±2.35	129.79±5.64	116.79±3.98	109.62±3.51
	T_0	**T_1**	**T_2**	**T_0**	**T_1**	**T_2**
Studies	**Serum ALP (IU/L)**			**Bilirubin (mg/dL)**		
Study I	166.19±7.26	165.16±7.56	163.72±7.23	0.79±0.02	0.75±0.04	0.70±0.01
Study II	261.61±10.45	234.75±5.87	220.9±8.26	1.35±0.08	1.22±0.06	1.18±0.02
Study III	250.46±11.65	223.63±7.56	219.91±8.21	1.16±0.02	1.05±0.05	1.01±0.01
Study IV	302.09±12.54	283.09±11.54	272.88±10.65	1.40±0.09	1.30±0.07	1.23±0.04

superoxide and hydroxyl radicals in vitro. The anti-oxidant mechanism of aqueous date palm extract may be related to the ability of its active compounds to detoxify free radicals and to inhibit lipid per-oxidation in the liver. Anti-inflammatory effect of polyphenols is also demonstrated by its ability to inhibit the production of nitric oxide and tumor necrosis factor a (TNF-a) (51).

Similar trend of AST reduction (39.01 & 53.10%) was observed by (34) in diabetic rats fed with 10 & 15% date pit powder supplemented bread, respectively. Later, (10) documented 2.99% reduction in serum AST level at feeding regime of 70g/kg of diet. However, (49) noticed 46.91% reduction for this parameter in rats with thioacetamide-Induced hepatotoxicity. Similar effect of AST reduction (40%) was reflected from the findings of (48) after administering 4ml/kg body weight to rats with dimethoate induced oxidative stress. Earlier, (52) observed similar diminution trend in AST level in rats with carbon tetrachloride induced oxidative stress. Likewise, (53) observed 1.80% reduction in AST level by giving daily ingestions of 10 ml of date pit extract for 8 weeks.

Similarly, (29) carried out research to explore the effect of date palm extract on ALP level by feeding male albino rats with date palm extract for 30 days at the rate of 10, 20, 30 and 40mg/100g diet and recorded progressive decrease in the blood ALP level as 5.04, 12.12, 15.71 and 18.22%, respectively

Recently (46) narrated 13.93% decreasing trend in serum ALP level of normal and lead acetate induced oxidative stress rats after consuming 300 mg/kg body weight of Ajwa date extract. The detected significant reduction in the serum enzymes level by Ajwa extract could be attributed to a decrease in the lipid peroxidation of hepatocellular membrane induced by the production of reactive oxygen species due to high cholesterol diet. Also, it may be due to the accelerated regeneration/repairing of damaged hepatocytes. A similar conclusion was clarified by (48, 54).

The instant outcomes are in line with the studies on male wistar rats conducted by (10) proved that date pits reduce bilirubin level owing to the presence of high number of antioxidants. They fed male Wistar rats with a basal diet containing 70g/kg date pits for 30 days, it was observed that date pits significantly reduced blood bilirubin level by 17.83%. Likewise, (9) narrated 25.09% decline in the bilirubin level of normal and carbon tetrachloride (CCl_4) induced oxidative stress rats after consuming 1g/kg body weight Ajwa date extract.

Conclusion

The drink containing 10% Ajwa roasted date pit powder was more effectual in reducing cholesterol, LDL, triglycerides, glucose levels and increasing HDL and insulin levels than drink containing 5% Ajwa roasted date pit powder. It is executed that Ajwa date pit be endowed with vivid forthcoming to ameliorate the effect of oxidative stress, thus stopping the chain reactions implicated in the onset of chronic diseases. The outcomes of current project found Ajwa date pit most effective against obesity and allied discrepancies including hyperglycemia and hypercholesterolemia.

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