

Effect of Palm Date Seeds Powder and Extract on Hypercholesterolemic, and Diabetic Male Albino Rats

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Abstract:

This study was carried out to determine the effect of mature date kernel powder on males of white mice weighing 150-200 g. Rats were divided into eight groups (two for control "-" groups) and two control (+) groups inflicted with hypercholesterolemia and diabetes mellitus were fed on the basal diet with germinated powdered dates stones 5%, and two groups also infected with hypercholesterolemia and high blood sugar are fed basal feed with extracts from dates soaked in methanol, dried and added to distilled water after methanol volatilization are administered orally at 1/2 ml level daily.

High cholesterol in serum of male rats caused by feeding them on the standard diet containing 1.5% cholesterol plus 10% fat from sheep tail for 15 days according to **Ain (1993)**.

Diabetic rats were previously injected with alloxan at 150 mg / kg body weight into the peritoneal membrane according to method described by **Desai and Bhide (1985)**.

At the end of the experiment (28 days) body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) calculated and the blood samples, were taken from ventricular vein to serum separate for biochemical analysis. Slaughtering carried out for rats after 12 hours of fasting under anesthesia. Blood samples were taken in dry tubes and left to coagulate at room temperature, then centrifuged for 10 min at 3000 rpm to separate the serum. The serum was aspirated

carefully and transported with clean cuvette tubes. serum samples were Kept for analysis until in the freezer at - 20°C (Schichmer, 1967).

The biological parameters are included (BWG, FI & FER).Kidneys function evaluated by determining creatinine,urea &uric acid. lipids profile of serum also evaluated. Liver function parameters included(AST, ALT, ALP) and (bilirubin) serum glucose used as marker for diabetes intensity.

The best results of the study with recorded for groups trusted with the date palm pits extract 1/2 ml. Mean while palm date kernel powder showed also marked improvement of renal function and hypercholesterolemia and reduced their side effects.

Keywords: Palm date kernel. hypercholesterolemia, diabetes mellitus, rats.

INTRODUCTION

The date palm is largely grown in arid and semiarid regions of the world. World production of date seeds reached approximately 863,000 t in 2004. Date seeds are being wasted in large quantities or used mainly for animal (camels, cattle, sheep and poultry) feed. In addition, date seeds are used on a very limited scale in making a caffeine-free beverage with a coffee-like flavour. (Hussein *et al.*, 1998; FAO, 2004; Rahman *et al.*, 2007; Habib and Ibrahim 2008, 2011).

Antioxidant activity is closely related to the phenolic content of plants. Because synthetic antioxidants such as butylated hydroxytoluene or butylated hydroxyanisole; could promote cancer development in rats, and the fact that consumers are much interested in natural food additives, herbal phenolic compounds and other natural antioxidants are extremely desirable. Some of these compounds were found in date seed oil: Hydroxytyrosol, protocatechuic acid, tyrosol, gallic acid, caffeic acid, p-coumaric acid and oleuropein (Ardekani *et al.*, 2010). According to some authors Phoenix dactylifera extract has antibacterial effect against *E. fecalis*, indicating that this extract can be used for treating enteric diseases. Also, an important report of extracts of the flesh and pits of Phoenix dactylifera in gentamicin treated nephrotoxicity rat model showed significantly reduced increase in plasma creatinine and urea concentrations and ameliorated the proximal tubular damage (Rahmani *et al.*, 2014). Date seed extract (DSE), especially from germinated seeds, increases serum paraoxonase and arylesterase activities. These beneficial effects may be due to the presence of natural antioxidants such as phenolic compounds in the palm date seeds (Takaeidi *et al.*, 2014) Germination of palm date seeds was said to improve the biological effect of seeds (Juan *et al.*, 2016 & Burghardt *et al.*, 2016).

MATERIALS AND METHODS

The used plants and their preparation:

Palm date kernel (seeds) were washed well to get rid of any foreign material. Date pits placed between two layers of cloth wetted by a spray of clean water twice a day for a month at room temperature 20-26°C during the winter. Germinated seeds (1cm), dried in direct sunlight, taking into account to be removed and kept during the night in order to prevent dirt & rain. Then germinated & dried seeds milled in electrical mill to fine powder and placed in a plastic bag at chilling

temperature. The powder mixed with methanolic alcohol at 5% level, shakes at 60°C for 12 hours., filtrated, centrifugated at 400 rpm for 10 min, and alcohol repelled in a rotary evaporator, at 60°C. The powdered extract, which was diluted with distilled water and used in the by oral injection at a rate of 1/2 ml rat. Also, date seeds powder (unextracted) mixed with the basal diet to be used in feeding the rats (powder treatment).

Experimental animals.

Fifty six (56) adult male albino rats, weighting 150-200 g were used in this study.

Basal diet composition of tested rats

Basel diet composition of tested rats was according to **AIN (1993) and Reeves *et al.*, (1993)**. It was consisted of 15% protein (casein), 10% sucrose, 10% corn oil, 0.2% choline chloride, 1% vitamin mixture, 4% salt mixture and 5% fiber (cellulose); the remainder was corn starch. The composition of vitamin mixture and salt mixture were as recommended by (**Hegsted *et al.*, 1941) and Campbell (1963)** respectively.

Experimental design:

The male albino rats obtained from Agriculture Research Center, Giza, Egypt. Rats were housed in wire cages under the normal laboratory condition, and were fed on basal diet for a week as an adaptation period. Diet was offered to rats in special feed cups to avoid loser conditions of feeds. Water was provided to the rats by glass tubes supported to one side of the cage, feed and water provided ad libium and checked daily.

Hypercholesterolemia was induced in normal healthy male albino rats by feeding on diet containing 1.5 % cholesterol plus 10 % sheep tail fat for 15 days according to the method described by **Ain (1993)**.

Diabetes was induced in normal healthy male albino rats by intra peritoneal injection of alloxan 150 mg /kg body weight, according to the method described by **Desai and Bhide (1985)**.

Each group consisted of seven rats.

Grouping design and feeding of rats:

Groups of control (-) rats: Healthy rats fed on basal diet only.

Groups of control (+) rats: Inflicted rats with either hypercholesterolemia or diabetes mellitus and fed on basal diet only.

Groups of palm date powder diet: Inflicted rats with either hypercholesterolemia or, diabetes mellitus and fed on basal diet with palm date powder 5%.

Groups of palm date extract diet: Inflicted rats with either hypercholesterolemia, or diabetes mellitus and fed with 1/2 ml extract.

Biological evaluation:

During the experimental period (28 days), the diet consumed diet (FI) was recorded every day, and body weight recorded every week. The body weight gain (BWG), feed efficiency ratio (FER), were determined according to **Chapman *et al.*, (1959)**. Using the following equations:

BWG (g) = Final weight - Initial weight.

$$\text{FER} = \frac{\text{Grams gain in body weight (per day) } y}{\text{Grams feed consumed (per day) } y}$$

Biochemical evaluation:

Blood samples were collected after 12 hours fasting at the end of the experiment using the abdominal aorta where the fasting rats were sacrificed under ether anesthesia. Blood samples were received into clean dry centrifuge tubes and left to clot at room temperature, then centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum was carefully aspirated, transferred in to clean cuvette tubes, and stored frozen at -20C for analysis (**Schermer 1967**). All serum samples were analyzed for determination of the different parameters:

Estimation of creatinine in serum Creatinine was determined according to kinetic method of (**Henry 1974**). **Determination of uric acid:**

Serum uric acid was determined colorimetrically according to the method of (**Barham and Trinder, 1972**).

Determination urea nitrogen:

Urea was determined according to the enzymatic method of (**Patton and Crouch 1977**).

Estimation of serum lipids profile:

Triglycerides (T.G):

Serum TG were determined according to the method described by (**Fossati and Principe 1982**).

Total cholesterol (TC):

Serum total cholesterol (TC) was determined according to the method described by (Allain 1974).

Determination of high density lipoprotein cholesterol (HDL):

Serum HDL-c was colorimetrically determined according to the method described by (Fendewaid, 1972 & Grodon and Amer, 1977).

Determination of low density lipoprotein cholesterol (LDL):

Serum low density lipoprotein cholesterol (LDL-c) was calculated as mg/dl according to (Castelli et al., 1977) equation:

$$\text{LDL concentration mg/dl} = \text{Total Cholesterol} - \text{HDL} - \text{VLDL}$$

Determination of very low density lipoprotein cholesterol (VLDL):

Serum very low density lipoprotein cholesterol (VLDL-c) was calculated as mg/dl according to (Lee and Nieman 1996) equation:

$$\text{VLDL (mg/dl)} = \text{Triglycerides} / 5$$

$$\text{TG} = \text{VLDL} - \text{C Concentration (mg/dl)} = \text{TG} / 5$$

Calculation of atherogenic index (AI):

Atherogenic index was calculated according to (Nakabayashi *et al.*, 1995).

Determination of liver function parameters:

Determination of alkaline phosphatase (ALP):

ALP activities were measured in serum using the modified kinetic method (or liquicolor) of (Moss, 1982) by using kit supplied by Human, Germany.

Determination of aspartate transferase (AST):

AST activities were measured in serum using the modified kinetic method of (Henry, 1974).

Determination of (ALT):

Serum ALT was determined according to the method of (Tietz, 1976).

Direct Bilirubin:

Direct bilirubin also was determined in the serum according to the method described by Chary and Sharma (2004).

Determination of Serum glucose:

Serum glucose was measured using the modified kinetic method according to (Kaplan, 1984).

described by (Chary and Sharma, 2004).

Histopathological investigation:

At end of the experiment which continued for 28 successive days, all rats were sacrificed, Autopsy samples were taken from the internal

organs of rats and fixed in 10% buffered formalin for twenty-four hours. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin (**Banchroft et al., 1996**) for histopathological examinations by the light microscope.

Statistical analysis of data:

Data were statistically analyzed using a computerized costat program at the Scientific Computer Center, Faculty of Home Economics, Menofia University, using Duncan Multiple Range Test (one - way ANOVA test) according to (**Armitage and Berry, 1987**). The results are presented as mean \pm SD. Differences between treatments at ($P > 0.05$) were considered significant.

RESULTS AND DISCUSSION

1- Hypercholesterolemia:

a- BIOLOGICAL PARAMETERS

a- Effect of palm date kernel (*Phoenix dactylifera* L) powder and extract on body weight gain (BWG) feed intake (FI) and feed efficiency ratio (FER) of hypercholesterolemia rats:

1-Body Weight Gain (BWG):

Table (1) results show the effect of palm date kernel powder and extract on body weight gain of hypercholesterolemic rats. It could be observed that the mean BWG value of control (+) group was lower than control (-) group being 0.96 ± 0.03 & 1.97 ± 0.02 g respectively which revealed significant difference with percent increase 105.20% of control (-) group as compared to control (+). Other groups indicated significant differences as compared to control (+) group. The values were 1.9 ± 0.2 & 2.1 ± 0.2 g for palm date kernel powder and extract respectively. The percent of increases were 97.91% & 118.75% for groups 3,4 respectively. Groups 1 & 3 showed nonsignificant differences between them. The best body weight gain numerically recorded for group 4 (palm date kernel extract 1/2 ml) for hypercholesterolemic rats even when compared to control (-) group.

2-Feed Intake (g/day for each rat):

Table (1) results show the effect of palm date kernel powder and extract on feed intake of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was lower than control (-) group, being 15 ± 1 & 22 ± 2 g respectively which revealed significant

difference with percent of increase 46.66 % of control (-) group as compared to control (+). Other groups indicated significant differences as compared to control (+) group. The values were 18 ± 2 & 19 ± 2 g for palm date kernel powder & extract groups respectively. The percent of increases were 20 & 26.66 respectively for the abovementioned of groups. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for feed intake of hypercholesterolemic rats, but some lower when compared to control (-) group.

3-Feed Efficiency Ratio (FER): Table (1) illustrate the effect of palm date kernel powder and extract on feed efficiency ratio of hypercholesterolemic rats. It could be shown that the mean value of control (+) group was lower than control (-) group being 0.064 ± 0.002 & 0.09 ± 0.002 respectively which indicated significant difference with percent of increase 40.62 % of control (-) group as compared to control (+). Other two groups showed significant differences as compared to control (+) group. The values were 0.106 ± 0.002 & 0.111 ± 0.001 for palm date kernel powder and extract respectively. The percent of increase were 65.62 & 73.43% respectively for the abovementioned of groups. Group 4 (palm date kernel extract 1/2 ml) revealed the best group for feed efficiency ratio of hypercholesterolemic rats even when compared to control (-) group.

Table (1): Body weight gain (BWG g/day), feed intake (FI g/day) and feed efficiency ratio (FER) of Hypercholesterolemic rats as affected by feeding on diets containing 5% powder & 1/2 ml extract of palm date kernel

Parameters Group	BWG(g/day)	FI (g/day)	FER ratio
	Mean±SD	Mean±SD	Mean±SD
Group 1 Control- ve	1.97a±0.02	2±22a	0.09c±0.002
Group 2 Control+ ve	0.03±b 0.96	1±d 15	0.002±0.064d
Group 3 Powder 5%	0.2±1.9a	18c±2	0.002±0.106b
Group 4 Extract 1/2ml	0.2±2.1a	2±19b	0.001±0.111a
LSD	0.202	0.99	0.004

Means in the same row with different litters are significantly different and vice versa, Significance at ($p \leq 0.05$).

C- Effect of palm date kernel (Phoenix dactylifera L.) powder and extract on Lipids fractions of Hypercholesterolemia rats:

1- Total cholesterol (T.C):

Table (2) show the effect of palm date kernel (powder& extract) on Total Cholesterol of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 79 ± 1 & 61 ± 4 mg/dl respectively which illustrated significant difference with percent of decrease -22.78 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 77 ± 2 & 57 ± 2 mg/dl for palm date kernel powder & extract respectively. The percent of decrease were -2.53 & -27.84 % for the abovementioned groups. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for total Cholesterol of hypercholesterolemic rats even when compared to control (-) group.

2- Triglycerides

Table (2) show the effect of palm date kernel (powder& extract) on triglycerides of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 45 ± 4 & 42 ± 2 mg/dl respectively which illustrated significant difference with percent of decrease -6.66 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 36 ± 4 & 30 ± 3 mg/dl for palm date kernel powder & extract respectively. The percent of decrease were -20 & -33.33 % for the abovementioned groups respectively. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for Triglycerides of hypercholesterolemic rats even when compared to control (-) group.

Table (2) show the effect of palm date kernel (powder& extract) on VLDL (mg/dl) of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 9 ± 3 & 8 ± 4 mg/dl respectively which illustrated significant difference with percent of decrease -11.11 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 7 ± 4 & 6 ± 3 mg/dl for palm date kernel powder& extract respectively. The percent of decrease were -22.22 & -33.33 % for the abovementioned groups respectively. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for VLDL (mg/dl) of hypercholesterolemic rats even when compared to control (-) group.

4- High density lipoprotein cholesterol (HDL) in serum:

Table (2) show the effect of palm date kernel (powder& extract) on HDL of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was lower than control (-) group, being 42 ± 2 & 43 ± 3 mg/dl respectively which illustrated significant difference with percent of decrease -2.38% of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 47 ± 4 & 54 ± 3 mg/dl for palm date kernel powder& extract respectively. The percent of decrease were 11.90 & 28.57 % for the abovementioned groups. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for HDL of hypercholesterolemic rats when compared to control (+) group.

5- Low density lipoprotein cholesterol in serum (LDL):

Table (2) show the effect of palm date kernel (powder& extract) on LDL of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 28 ± 3 & 10 ± 3 mg/dl respectively which illustrated significant difference with percent of decrease -64.28% of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 23 ± 3 & 3.0 ± 2 mg/dl for palm date kernel powder& extract respectively. The percent of decrease were - 17.85 & -89.28 % for the abovementioned groups. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for LDL of hypercholesterolemic rats even when compared to control (-) group.

6- Atherogenic index (AI):

Table (2) show the effect of palm date kernel (powder& extract) AI(U-L) of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 0.9 ± 0.2 & 0.4 ± 0.2 ratio respectively which illustrated significant difference with percent of decrease -55.55 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 0.6 ± 0.3 & 0.2 ± 0.1 ratio for palm date kernel powder& extract respectively. The percent of decrease were - 33.33 & -77.77% for the abovementioned groups. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for AI (ratio) of hypercholesterolemic rats even when compared to control (-) group.

Table (2): Serum Total cholesterol (T.C), Triglycerides, (TG), VLDL(mg/dl), High density lipoprotein cholesterol (HDL), Low density lipoprotein cholesterol in serum (LDL) & Atherogenic index (AI) ratio of hypercholesterolemia rats as affected by feeding on diets containing of 5% powder & 1/2 ml extract palm date kernel

Parameters Group	T.C (mg/dl) Mean±SD	T.G (mg/dl) Mean±SD	VLDL(mg/dl) Mean±SD	HDL(mg/dl) Mean±SD	LDL(mg/dl) Mean±SD	AI (ratio) Mean±SD
Group 1 Control-ve	61 ±4 b	42 ± 2 b	8±4ab	43 ± 3 c	10 ±3 c	0.4 ±0.2 c
Group2 Control+ ve	79 ±1 a	45 ±4 a	9 ±3a	42 ±2 c	28±3 a	0.9 ±0.2 a
Group3 Powder 5%	77±2 a	36±4 c	7 ± 4 bc	47±4 b	23 ±3 b	0.6±0.3 b
Group4 Extract 1/2 ml	57±2 c	30 ±3 d	6 ±3c	54±3 a	3.0±2 d	0.2±0.1 d
LSD	2.513	1.912	1.153	1.631	0.99	0.163

Means in the same row with different litters are significantly different and vice versa, significance at ($p \leq 0.05$).

Coronary heart disease is the leading cause of death among Americans, and the oxidation of low density lipoproteins (LDL) plays a key role in the development of coronary heart disease. studies evaluated the capacity of polyphenolic antioxidants in certain Asian vegetables and California dates to inhibit LDL oxidation and potentially reduce heart disease risk. Samples of cilantro, bok choy, Kraton bok, a vegetable grown and consumed primarily in Thailand, and California dates were obtained. All samples were dried, then ground into a fine powder. Polyphenols were extracted from each sample by methanol extraction food polyphenols have been shown to be potent antioxidants, and appear to be able to inhibit LDL oxidation which is linked to coronary heart disease risk. This study found the Thai vegetable Kraton bok was rich in polyphenols, and highly effective for inhibiting LDL oxidation, while dates were found to have the lowest polyphenol content, and to be least effective inhibiting LDL oxidation. Additional information on the polyphenolic content of food, and their antioxidant capacity is needed before the relative importance of polyphenols in date stones also reducing coronary heart disease can be fully appreciated. (Hughes and Ganthavorn, 2009).

Generally, various type of date palm have different physicochemical properties including antioxidant activity, dry matter and total phenolics contents. Total phenolics assay may be sufficient to evaluate antioxidant properties in date fruit. In the present study, ethanol extract of Zardan bears comparable phenolics group and

antioxidant activity to the standard compounds. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases. (Ebrahimzadeh et al., 2008). The suitable antioxidant activity of date palm supports its possible use as a natural antioxidant in food industries and other pharmaceutical preparations. (Zahra Sadeghil *et al.*, 2015).

Effect of palm date kernel(*Phoenix dactylifera* L) powder and extract on Liver function parameters of Hypercholesterolemic rats a-AST liver Enzyme In Serum (u/L).

Table (3) results show the effect of palm date kernel (powder& extract) on AST of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 118 ± 2 & 58 ± 3 u/l respectively which illustrated significant difference with percent of decrease -54.23% of control (-) group as compared to control (+). All other groups show significant differences as compared to control (+) group. The values were 90 ± 5 & 55 ± 5 u/l for palm date kernel powder& extract respectively. the percent of decrease were -23.72& -53.38 % for the abovementioned groups. Group 4(palm date kernel extract 1/2 ml) recorded the best group for AST of hypercholesterolemic rats when compared to control (+) group.

b-ALT liver Enzyme In Serum (U/L):

Table (3) results show the effect of palm date kernel (powder& extract) on ALT of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 41 ± 4 & 32 ± 3 u/l respectively which illustrated significant difference with percent of decrease -21.95% of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 36 ± 3 & 31.33 ± 2.51 u/l for palm date kernel powder& extract respectively. The percent of decrease were -12.19& -23.58 % for the abovementioned groups. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for AST of hypercholesterolemic rats even when compared to control (+) group.

c- ALP activity.

Table (3) results show the effect of palm date kernel (powder& extract) on ALP (U/L) of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 360 ± 3 & 211 ± 4 u/l respectively which illustrated significant difference with percent of decrease -41.38% of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 155 ± 5 & 152 ± 4 u/l for palm date kernel powder& extract respectively. The percent of decrease were -56.94 & -57.77% for the abovementioned groups. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for ALP (U/L) of hypercholesterolemic rats even when compared to control (+) group.

Table (3): Serum AST, ALT, ALP & AST/ALT of hypercholesterolemia rats as affected by feeding on diets containing of 5% powder & 1/2 ml extract palm date kernel

Parameters Group	AST(U/L) Mean±SD	ALT (U/L) Mean±SD	ALP(U/L) Mean±SD
Group 1 Control-ve	4 ^c ± 54	3 ^c ±32	4 b±211
Group2 Control+ ve	2 a±118	4 a±41	3 a±360
Group3 Powder 5%	5 b±90	3 b±36	5 c±155
Group4 Extract 1/2 ml	5 ^c ±55	2.51 c±31.3	4 d±152
LSD	2.82	1.28	1.63

Means in the same row with different litters are significantly different and vice versa,significance at ($p \leq 0.05$).

Palm date fruits have been used extensively for the traditional cure of liver illnesses and malaria in the Arab Peninsula. The in vivo hepatoprotective and the antioxidative effects of different varieties of palm date fruits and stones in relation to their total phenolic contents and total flavonoids were studied. The antioxidative and hepatoprotective effects of palm date syrups were very clear, since results of plasma ALT and AST and TBARS (from liver homogenate) of the control were significantly higher than those obtained from animals treated with syrups. The present findings do strongly recommend to increase the consumption of palm date fruits and stones, especially in cases of liver diseases and for the prevention of other

serious diseases such as cardiovascular and cancers. (Al-Shoaibi *et al.*, 2012).

Effect of palm date kernel (*Phoenix dactylifera* L) powder and extract on some renal function parameters of Hypercholesterolemia rats

1-Serum Creatinine (mg/dl):

Table (4) Show the effect of palm date kernel (powder& extract) on creatinine (mg/dl) of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 0.5 ± 0.2 & 0.4 ± 0.2 mg/dl respectively which significant difference with percent of decrease -20% of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 0.4 ± 0.3 & 0.3 ± 0.2 mg/dl for palm date kernel powder& extract respectively. The percent of decrease were -20 & -40 % for the abovementioned groups compared to control (+) rats. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for creatinine of hypercholesterolemic rats even when compared to control (-) group.

2- Uric Acid in serum:

Table (4) Show the effect of palm date kernel (powder& extract) on Uric acid (mg/dl) of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 1.5 ± 0.3 & 1.3 ± 0.2 mg /dl respectively which illustrated significant difference with percent of decrease -13.33 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 1.3 ± 0.2 & 1.1 ± 0.2 mg/dl for palm date kernel powder& extract respectively. The percent of decreases were -13.33 & -26.66 % for the abovementioned groups. Group 1&3 showed nonsignificant differences between them.

Group 4 (palm date kernel extract 1/2 ml) recorded the best group for uric acid of hypercholesterolemic rats when compared to control (-) group.

3- Blood Urea Nitrogen (mg/dl):

Table (4) Show the effect of palm date kernel (powder& extract) on BUN(mg/dl) of hypercholesterolemic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 12 ± 2 & 11 ± 3 mg/dl respectively which illustrated significant difference with percent of decrease -8.33 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 11 ± 3 & 11 ± 2 mg/dl for palm date kernel powder& extract respectively. The percent of decrease were -8.33&-8.33 %for the abovementioned groups. Group 4 (palm date kernel extract 1/2 ml) record' lcv tyed the best group for BUN of hypercholesterolemic rats even when compared to control (-) group.

Table (4): Creatinine, Uric acid & B U N of hypercholesterolemia rats as affected by feeding on diets containing 5% powder & 1/2 ml extract of palm date kernel

Parameters Group	Creatinine (mg/dl) Mean±SD	(mg/dl) Uric acid Mean±SD	B U N (mg/dl) Mean±SD
Group 1 Control-ve	0.4±0.2 b	1.3±0.2 b	11 ± 3b
Group2 Control+ ve	0.5±0.2 a	1.5±0.3 a	12 ± 2a
Group3 Powder 5%	0.4±0.3 b	1.3±0.2 b	11 ± 3b
Group4 Extract 1/2 ml	0.3±0.2 c	1.1±0.2 c	10.5 ± 2c
LSD	0.09	0.09	0.53

Means in the same row with different litters are significantly different and vice versa, significance at ($p \leq 0.05$).

Date seeds have previously been examined for extractable high value-added components for incorporation into functional foods. The results showed that date seeds contained large quantities of fiber, and possibly resistant starch, which may have potential health benefits. (Mohammad *et al.*, 2010). Date seeds have been shown to contain high amounts of antioxidants. suggesting a protective of date seeds against in vivo oxidative damage, possibly through the action of their bioactive antioxidants.(Habib & Ibrahim, 2011). Oxidative change are damaging to renal function. (Allaith *et al.*, 2012).

Effect of palm date kernel(Phoenix dactylifera L.) powder and extract on protein fraction (Direct Bilirubin) of Hypercholesterolemic rats

1- Direct Bilirubin (mg/dl):

Table (5) show the effect of palm date kernel (powder& extract) on direct bilirubin (D.Bil) of hypercholesterolemic rats. It could be noticed that the mean D.Bil value of control (+) group was higher than control (-) group, being 1.49 ± 0.2 & 0.33 ± 0.1 mg/dl respectively which illustrated significant difference with percent of decrease -77.85 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 0.65 ± 0.3 & 0.48 ± 0.1 mg/dl for palm date kernel powder& extract respectively. The percent of decrease were -56.37% & -67.78% for the abovementioned groups. Group 4 (palm date kernel extract 1/2ml) recorded the best group for Direct bilirubin of hypercholesterolemic rats even when compared to control (+) group.

Table (5): Direct Bilirubin (mg/dl) of hypercholesterolemic rats as affected by feeding on diets containing 5% powder & 1/2 ml of extract of palm date kernel

Group	Parameters	Direct Bilirubin (mg/dl)	
		SD±Mean	%Change of positive Control
Group 1 Control-ve		0.33 ± 0.1 d	-77.85
Group2 Control+ ve		1.49 ± 0.2 a	-----
Group3 Powder 5%		0.65 ± 0.3 b	-56.37
Group4 Extract 1/2 ml		0.48 ± 0.1 c	-67.78
LSD			0.02

Means in the same row with different litters are significantly different and vice versa, Significance at ($p \leq 0.05$).

It was shown that the date pits extract (water: methanol: acetone: formic acid) had significantly the highest levels of total polyphenols and antioxidant activity. Also, the obtained results indicated that phenolic compounds in date pits of khalas variety had high antioxidative effect in reducing the formation of hydroperoxides during storage. (Basuny *et al.*, 2011).

The role of date seed extract (DSE) in protection against cerebral ischemic damages was found previously. Treatment with D S E attenuated all of alterations and neuronal damage induced by middle cerebral artery occlusion in male rats. The data showed this treatment with could protect cortical neurons against cerebral-induced injuries

most probably due to its antioxidant properties (**Kalantaripour *et al.*, 2012**)

Date seed extract showed significant antioxidant activities in olive oil. The degrees of influence of the extracts were different and it could be change because of the chemical structure and olive oil'own antioxidant capacity. (**Mehmet and Fahad, 2015**)

11- Diabetes Mellitus:

A- BIOLOGICAL PARAMETERS:

a-Effect of palm date kernel (Phoenix dactylifera L) powder and extract on body weight gain(BWG g) feed intake (FI) and feed efficiency ratio (FER) of Diabetic rats:

1-Body Weight Gain (BWG g):

Table (6) show the effect of palm date kernel powder and extract on body weight gain in Diabetic rats. It could be observed that the mean value of control (+) group was lower than control (-) group being 1.19 ± 0.01 & 2.24 ± 0.02 g respectively which revealed significant difference with percent of decrease 88.23 % of control (-) group as compared to control (+). All other groups indicated significant differences as compared to control (+) group. The values were 1.75 ± 0.02 & 1.88 ± 0.01 g for palm date kernel powder and extract respectively. The percent of increases were 47.05 & 57.98 respectively for the aforementioned groups. Group 4(palm date kernel extract 1/2 ml) recorded the best group for body weight gain of Diabetic rats when compared to control (+) group

2-Feed Intake (g/day for each rat):

Table (6) show the effect of palm date kernel powder and extract on feed intake by Diabetic rats. It could be noticed that the mean value of control (+) group was lower than control (-) group, being 15 ± 2 & 23 ± 2 g respectively which revealed significant difference with percent of increase 53.33 % for control(-) group as compared to control (+). All other groups indicated significant differences as compared to control (+) group. The values were 17 ± 1 & 18 ± 1 g for palm date kernel powder and extract respectively. The percent of increase were 13.33 & 20% respectively for the abovementioned of groups respectively. Groups (3&4) show nonsignificant differences between

them. Group 4(palm date kernel extract 1/2 ml) recorded the best group for feed intake of Diabetic rats when compared to control (+) group.

3-Feed Efficiency Ratio (FER):

Table (6) illustrate the effect of palm date kernel powder and extract on feed efficiency ratio in Diabetic rats. It could be shown that the mean value of control (+) group was lower than control (-) group being 0.079 ± 0.002 & 0.097 ± 0.001 g respectively which indicated significant difference with percent of increase 22.78 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 0.103 ± 0.001 & 0.104 ± 0.001 g for palm date kernel powder and extract respectively. The percent of increases were 30.37 & 31.64% respectively for the abovementioned of groups respectively. Group 4(palm date kernel extract 1/2 ml) revealed the best treatment for feed efficiency ratio of diabetic rats even when compared to control (-) group.

Table(6): Body weight gain (BWG g/day), feed intake (FI g/day) and feed efficiency ratio (FER) of Diabetes Mellitus rats as affected by feeding on diets containing of 5% powder & 1/2 ml extract of palm date kernel

Parameters	BWG(g/day)	FI (g/day)	FER ratio
Group	Mean \pm SD	Mean \pm SD	Mean \pm SD
Group 1 Control- ve	2.24 ^a \pm 0.02	23 ^a \pm 2	0.097 ^c \pm 0.001
Group2 Control+ ve	1.19 ^d \pm 0.01	15 ^c \pm 2	0.079 ^d \pm 0.002
Group3 Powder 5%	1.75 ^c \pm 0.02	17 ^b \pm 1	0.103 ^b \pm 0.001
Group4 Extract 1/2ml	1.88 ^b \pm 0.01	18 ^b \pm 1	0.104 ^a \pm 0.001
LSD	0.011	1.15	9.98

Means in the same row with different litters are significantly different and vice versa, Significance at ($p \leq 0.05$).

Results published by (Hashim *et al.*, 2014) indicated, that the average body weight decreased due to diabetic mellitus of rats, as compared to normal control group. On the other hand, oral administration of different doses of P. Virgatus methanolic extract, and

its partially purified fraction showed significant gain in body weight of diabetic rats when compared control (+) group.

d-Effect of palm date kernel(*Phoenix dactylifera L.*) powder and extract on Serum Glucose of Diabetic rats.

Table (7) show the effect of palm date kernel powder and extract on Glucose in diabetic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 277 ± 3 & 161 ± 4 mg/dl respectively which illustrated significant difference with percent of decrease -41.87 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) groups. The values were 173 ± 3 & 143 ± 3 mg/dl for palm powder and extract respectively. The percent of decrease were date kernel -37.54 & -48.37 % for the abovementioned groups.

Group (4)(palm date kernel extract 1/2 ml) recorded the best group for Glucose of Diabetic rats even when compared to control (-) group

Most important, fasting serum glucose and triacylglycerol levels were not increased after consumption of either date variety. and Serum triacylglycerol levels even significantly ($p < 0.05$) decreased, by 8 or 15% after Medjool and Hallawi dates. Basal serum oxidative status was significantly ($p < 0.01$) decreased by 33% as compared to the levels observed before consumption In agreement with the above results, serum activity of the HDL-associated antioxidant enzyme paraoxonase 1 (PON1) significantly increased, by 8%, after Hallawi date consumption. (Rock *et al.*, 2009)

Date seed extract administration is safe on the liver and kidney. In addition, insulin-date seed extract combination minimizes the toxic effects of diabetes on these organs. (El Fouhil *et al.*, 2011).

Many authors confirmed our data of table (7) such as (EL Fouhil *et al.*, 2011). reported the safety of date seed extract administration to diabetic rats. (Mona Halaby *et al.*, 2014) found that fortified pan bread with date pits powder revealed decreased in serum the serum level glucose in diabetic rats.

Table(7): Glucose (mg /dl) of Diabetes mellitus rats as affected by feeding on diets containing of 5% powder & 1/2 m extract of palm date kernel

Parameters Group	Glucose(mg/dl)	
	SD±Mean	%Change of positive control
Group 1 Control -ve	161 ^c ± 4	-41.87
Group2 Control+ ve	277 ^a ± 3	-----
Group3 Powder 5%	173 ^b ± 3	-37.54
Group4 Extract 1/2 ml	143 ^d ± 3	-48.37
LSD		0.99

Means in the same row with different litters are significantly different and vice versa, significance at ($p \leq 0.05$).

B-BIOCHEMICAL PARAMETERS:

a-Effect of palm date kernel (*Phoenix dactylifera* L) powder and extract on Some renal function parameters of Diabetic rats.

1-Serum Creatinine (mg/dl)

Table (8) show the effect of palm date kernel (powder& extract) on creatinine (mg/dl) in Diabetic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 1.4 ± 0.2 & 0.5 ± 0.2 mg/dl respectively which illustrated significant difference with percent of decrease -64.28 % of control (-) group as compared to control (+). Other the values were 1.3 ± 0.2 & 1 ± 0.2 mg/dl for palm date kernel powder & extract respectively. The percent of decreases were -7.14 & -28.57 % for the abovementioned groups respectively. Group 4 (palm date kernel extract 1/2 ml) recorded the best group for creatinine of Diabetic rats when compared to control (+) rats.

2- Uric acid:

Table (8) show the effect of palm date kernel (powder& extract) on Uric acid (mg/dl) in Diabetic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 3.8 ± 0.4 & 1.6 ± 0.3 mg/dl respectively which illustrated significant difference with percent of decrease - 57.89 % of control (-) group as compared to control (+). All other groups showed significant

differences as compared to control (+) group. The values were 1.7 ± 0.2 & $\pm 1.4 \pm 0.2$ mg/dl for palm date kernel powder & extract respectively. The percent of decrease were -55.26 & -63.15% for the abovementioned groups.

Group 4 (palm date kernel extract 1/2 ml) recorded the best group for urea acid of Diabetic rats even when compared to control (-) groups respectively.

3- Blood Urea Nitrogen (mg/dl):

Table (8) show the effect of palm date kernel (powder & extract) on BUN (mg/dl) in Diabetic rats. It could be noticed that the mean value of control (+) group was higher than control (-) group, being 26 ± 3 & 21 ± 3 mg/dl respectively which illustrated significant difference with percent of decrease -19.23 % of control (-) group as compared to control (+). All other groups showed significant differences as compared to control (+) group. The values were 23 ± 2 & 22 ± 4 mg/dl for palm date kernel powder & extract respectively. The percent of decrease were -11.53 & -15.38 % for the abovementioned groups respectively. Group 4 (palm date kernel extract 1/2ml) recorded the best group for BUN of Diabetic rats when compared to control (+) group.

Table (8): Serum creatinine, Uric acid & B U N of Diabetes mellitus rats affected by feeding on diets containing 5% powder & 1/2 ml extra napalm date kernel

Parameters Group	Creatinine (mg/dl) Mean \pm SD	(mg/dl) Uric acid Mean \pm SD	B U N (mg/dl) Mean \pm SD
Group 1 Control-ve	0.5 ^c \pm 0.2	1.6 ^b \pm 0.3	21 ^b \pm 3
Group 2 Control+ ve	1.4 ^a \pm 0.2	3.8 ^a \pm 0.4	26 ^a \pm 3
Group 3 Powder 5%	1.3 ^a \pm 0.2	1.7 ^b \pm 0.2	23 ^b \pm 2
Group 4 Extract 1/2 ml	1 ^b \pm 0.2	\pm 0.2 1.4 ^c	22 ^c \pm 4
LSD	0.199	0.19 1	1.63

Means in the same row with different litters are significantly different and vice versa, significance at ($p \leq 0.05$).

Sheir, Marwa (2009) found that nephropathy increased serum creatinine, which was lowered by feeding on diets containing turmeric and ginger.

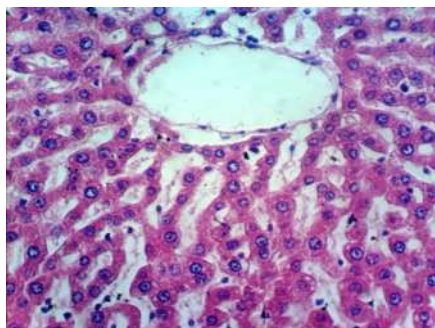
An important report of the flesh and pits extracts of (*Phoenix dactylifera* L) in gentamicin treated nephrotoxicity rat model showed

significantly reduction creatinine and urea concentrations and ameliorated the proximal tubular damage (**Rahmani *et al.*, 2014**).

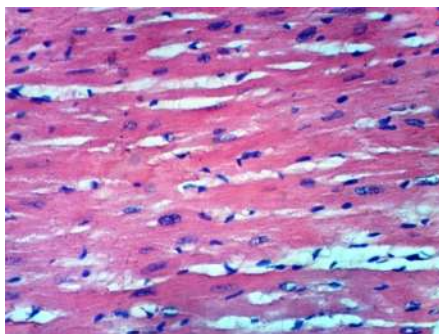
The Histopathological Results:

Liver section of normal rats (group 1) assigned for the diseases studies (diabetic, hypercholesterolemia rats).

Normal rats (control ' - ', basal diet) showed the normal histological structure of hepatic lobule from control vein and concentrically arranged hepatocytes (photo a)



photo(a): Liver of control, untreated rat control (-) (basal daiet) showing the normal histological structure of hepatic lobule from central vein and concentrically arranged hepatocytes (H & E X 400).



photo(b): Heart of rat from group 1 control (-) (basal diet) showing normal cardiac myocytes. (H & E X 400).

Hypercholesterolemic rats

Microscopically, liver of hypercholesterolemic rats from group (2) (control "+") (basal diet) revealed kupffer cells activation and binucleation of hepatocytes. also congestion of control vein and hepatic sinusoids (photo 3).

Nevertheless rats fed on 5% palm kernel diet powder revealed the normal unchanged histological structure of hepatic lobule (photo 4). and rats fed on palm kernel extract 1/2ml showed also the normal histological structure of hepatic lobule (photo 5).

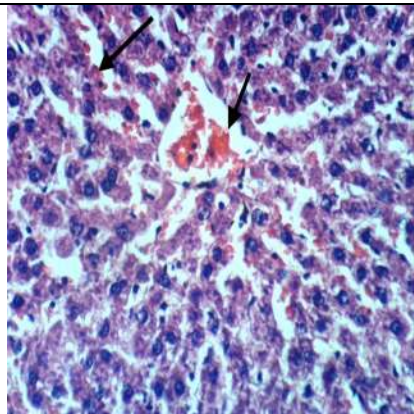


photo. (3): Liver of rat from (group 2) diabetes (control +) (basal diat) showing congestion of central vein and hepatic sinusoids (H & E X 400).

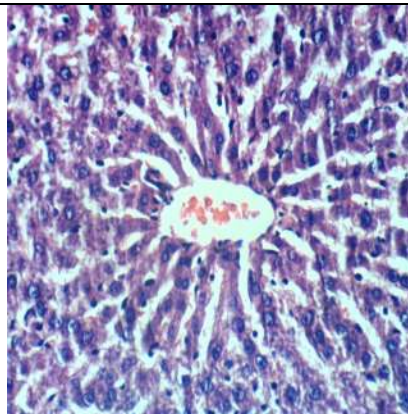


photo. (4): Liver of rat from (group 3) diabetes palm date kernel powder 5% showing the normal histological structure of hepatic lobule (H & E X 400).

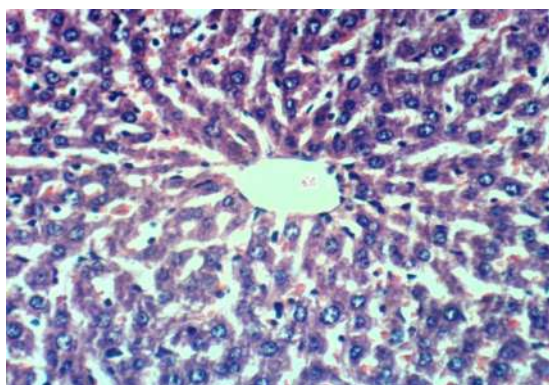


photo. (5): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).

Diabetic rats Microscopically diabetic rats of group 2 (control "+") (basal diet) revealed vacuolization of pancreatic acinar epithelium, necrosis of the cell of longer than, islets (photo 6)

On the other hand pancreas section of diabetic rats fed on palm kernel powder 5% (photo 7).or palm kernel extract 1/2ml (photo 8) revealed the normal histological structure of pancreatic parenchyma e.g. with no histological chores.

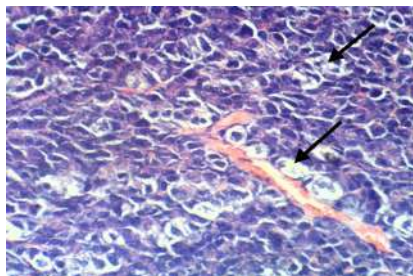


photo. (6): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).

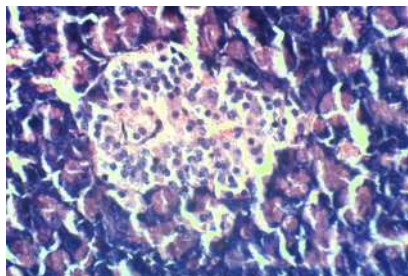


photo. (7): Pancreas of rat from group 2 showing no histopathological changes (H & E X 400).

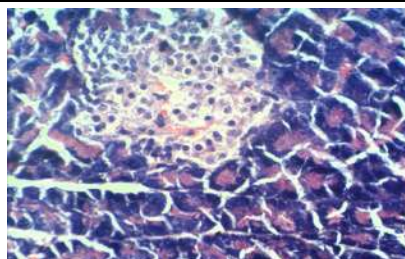


photo (8): Pancreas of rat from group 1 showing the normal histological structure of pancreatic parenchyma (H & E X 400).

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RECOMMENDATIONS

- 1- Much care should be directed to palm date kernels to be used for curing patients suffering of recent epoch diseases such as hypercholesterolemia, diabetes mellitus, each affecting at least 10% of Egyptians.
- 2- The effect of palm date seeds of different species on above diseases.
- 3- It is advised to pack palm date powder & extracts in gelatin capsules, after finding out the proper dose for humans in relation 20 disease, sex & age.
- 4- It is recommended to study the effect of palm date kernel powder as well as extract after in corporation in cake pan bread, cookies or sorp.
- 5- The remedial effect of palm date kernel powder water as well as organic solvents extracts for amelioration of iron deficiency anemia.

تأثير مسحوق ومستخلص انويه بلح النخيل على ذكور الفئران البيضاء المصابة بارتفاع الكوليسترول والسكرى

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رئيس بحوث الأغذية الخاصة والتغذية
مركز البحوث الزراعية، الجيزة، القاهرة

سحر محمد عبدالقادر ابراهيم عفيفى

المستخلص العربى

أجريت هذه الدراسة لتحديد تأثير مسحوق نواة التمر الناضجة على ذكور الفئران البيضاء التي تزن 150 - 200 جم،

مقسمة إلى ثمانية مجموعات (اثنان لمجموعات المقارنه -) واثنان لمجموعات المقارنه (+) للمرضين ومجموعتان (مجموعة للكوليسترول السيرم المرتفع ومجموعه للبول السكرى) قد تم تغذيتهما على الغذاء الاساسى المضاف اليه مسحوق نوى التمر 5% ومجموعتان ايضا مصابين بارتفاع الكوليستيرول وبالسكرى غذيت بالغذاء الاساسى مع متخلص نواة التمر بالفم 1/2 مل يوميا .

وقد تسببت التغذية بنسبة عالية من الكوليستيرول الخام لذكور الفئران البيضاء السليمه عن طريق اضافتها للغذاء الاساسى التي تحتوي على 1.5% من الكوليستيرول بالإضافة إلى 10% من دهون ذيل الخراف لمدة ثلاث شهور ادى لارتفاع الكوليستيرول فى السيرم . وقد تم اصابه ذكور الفئران البيضاء السليمه (بالحقن بالالوكسان 150 ملغم / كغم من وزن الجسم) بالغشاء البريتوني ادى لاصابه الفئران بالسكرى.

في نهاية التجربة (28 يوما) أخذت عينات الدم، بعد ذبح الفئران بعد 12 ساعة صوم وتحت تأثير التخدير بالاثير من الشريان الأورطي البطنى . تم اخذ عينات الدم فى انايب

جافه ونظيفه وتركت لتتجلط في درجة حرارة الغرفة، ثم الطرد المركزي لمدة 10 دقيقة على 3000 دورة في الدقيقة لفصل المصل وطرده تفاعلات الهواء من السيرم في انابيب . و نقلها بأنابيب كوفيت النظيفة خزن السيرم على درجه (-20) درجه مئوية لحين تحليل العينات تم تحليل المصل لتوضيح التأثيرات العلاجية المحتمله على مستويات الجلوكوز بالسيرم ودهون الدم ووظائف الكبد ووظائف الكلى و جزء البروتين (البيليروبين) عن طريق تجارب التغذية .

أفضل النتائج للدراسة لوحظت مع بذور نخيل التمر المنبته التي تم استخلاصها بمحلول بمعدل 2/1 مللى يوميا خلال فترة التجربه .علما بان مسحوق بذور البلح كان لها ايضا تأثير ملحوظ على الفيران المصابه بارتفاع الكوليستيرول بالسكرى .

الكلمات المفتاحيه : ارتفاع الكوليستيرول فى الدم - البول السكرى - مسحوق نوى البلح والمستخلص منه .