

***Therapeutic potential of low-fat yogurt supplemented with Dried Nabaq fruit (*Zizyphus spina-christi* L) on hyperglycemia in streptozotocin-induced diabetic rats.***

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***Abstract***

Nabaq (*Zizyphus spina-christi* L) fruit has a rich composition of bioactive components such as phenolic, flavonoids, dietary fiber, minerals and vitamins. The study was carried out to evaluate the effect of supplementation dried Nabaq fruit on the physicochemical, rheological and sensory properties of low fat yoghurt (1% fat). Dried Nabaq fruit was added to low fat milk yoghurt at ratios of 2 and 4 %.Results showed that gradual increase in pH, dietary fiber, viscosity, phenolic content and antioxidant activity values of low fat yoghurt as dried Nabaq fruit ratio was increased. On the other hand, the acidity and syneresis were decreased as the ratio of dried Nabaq fruit was increased. Low fat yoghurt that containing of 4% of dried

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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Nabaq fruit were the highest of physicochemical, acceptability sensory and rheological properties than the other treatments. Low fat yoghurt fortified with 4% dried Nabaq fruit were evaluated as hypoglycaemic agent streptozotocin-induced diabetic rats. Forty male rats were divided into 5 groups as follows: Group (1) non-treated non-diabetic rats (negative control). Group (2) diabetic rats (received Streptozotocin (STZ), 60 mg/Kg BW) (positive control). Group (3) diabetic rats treated with 20% low fat yoghurt without any additives.

Group (4) diabetic rats treated with 20% low fat yoghurt fortified with 4% dried Nabaq fruit. Group (5) diabetic rats treated with Meglitinides. The treatment of diabetic rats with low fat yoghurt containing dried Nabaq fruit showed a significant decreases ( $p < 0.001$ ) in levels of blood glucose, malondialdehyde (MDA), low density lipoprotein (LDL), cholesterol (CL), Triglyceride (TG), AST, ALT, ALP, Creatinin, Uric acid and Urea and increased ( $p < 0.001$ ) Insulin, high density lipoprotein (HDL) and total antioxidant capacity (TAC) in comparison to diabetic control rats. Thus, the study demonstrates that dried Nabaq fruit can be used as a source of phenolic compounds and dietary fiber in low fat yoghurt which enhanced its nutritional value, rheological and sensory properties and possess a hypoglycemia effect.

## ***Introduction***

Diabetes mellitus (DM) is a metabolic disorder that is characterized by an abnormal long-term increase in plasma glucose levels. Diabetes is mainly classified into four types, i.e., type I diabetes (T1DM), type II diabetes (T2DM), gestational diabetes, and specific types of diabetes due to other causes (***American Diabetes Association, 2019***).

Many factors, such as insulin deficiency or resistance as well as altered carbohydrate, protein, and fat metabolisms, are usually the reasons for high blood glucose levels leading to DM. Chronic hyperglycemia related to diabetes is often associated with many other complications, such as cardiovascular, dermatological, neurological, renal, retinal, and nerve diseases. Diabetes is one of the most common chronic diseases, and it has shown an increasing rate of occurrence over the past decade (***Bullard et al., 2018***).

According to the World Health Organization (WHO), the total number of people with diabetes worldwide substantially increased from 108 million in 1980 to 422 million in 2014 (***World Health Organization, 2016***). Along with diabetes, the incidence of other metabolic diseases, such as hyperlipidemia, is also increasing rapidly (***Karr, 2017***).

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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In type II diabetes mellitus, progressive decline in insulin action referred as insulin resistance and pancreatic  $\beta$ -cell dysfunction leads to increased levels of blood glucose (**Srinivasan *et al.*, 2005**). A major risk factor for insulin resistance is obesity, which is generally caused by a western style high fatty diet and physical inactivity (**Zheng *et al.*, 2012**).

The development of insulin resistance in obesity exhibits accelerated lipolytic activity with increased release of free fatty acids (FFA) into the portal circulation. The FFA may be cytotoxic by inducing lipid peroxidation and hepatocyte apoptosis resulting in fatty liver disease. Further, studies have shown that both obesity and type II diabetes impair insulin-induced suppression of glycogenolysis and gluconeogenesis. The current treatment for type II diabetes includes insulin and oral hypoglycemic drugs such as biguanides, sulfonylurea derivatives, thiazolidinediones and  $\alpha$ -glucosidase inhibitors. These medications have side effects, e. g. thiazolidinediones induce obesity, osteoporosis and sodium retention; sulfonylurea derivatives can lead to incidences of severe hypoglycemia, whereas biguanides like metformin put patients at risk of developing lactic acidosis (**Hamza *et al.* ,2010 and Bandawane *et al.* ,2020**).

Further, the oral monotherapy with lifestyle changes is not sufficient for most of the diabetic patients and requires various oral combinations or addition of insulin (**Stumvoll *et al.*, 2005**). Thus, there is an increasing need to search for effective antidiabetic agents

exhibiting fewer side effects. As an alternative, large number of population is trying to rely on plant-based remedies for management of this metabolic disorder. (**Bandawane et al., 2020**).

Free radical production is stimulated by hyperglycemia, leading to cell damage. In living organisms, oxidation is an essential process during the production of energy for use as fuel for biological processes. However, the increase in the formation of free radicals such as reactive oxygen species (ROS) plays a crucial role in the pathophysiology of several diseases such as diabetes, cancer, and cardiovascular disease. Oxidative stress may also play a role in metabolic syndrome, which is indicated by the presence of several oxidative biomarkers (**Končić et al., 2010**). In addition, the body's stress response may result in the progression or acceleration of numerous diseases related to oxidative stress (**Hemmati et al., 2016**).

Antioxidants can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or progression of oxidizing chain reactions. Previous studies have demonstrated that antioxidants can reduce markers of oxidative stress in both experimental and clinical models of diabetes. The redox status is disrupted in both type 1 and type 2 DM due to hyperglycemia, resulting in stress mediated cellular injury (**Hoshyar et al., 2015**).

Recently, consumers have trend to use of reduced fat dairy products but the functional role of fat in fermented milk causes enhancement of rheological and sensory characteristics (**Pakseresht et al., 2019**). However, since using plant natural additives in

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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production of low-fat fermented dairy products given some advantages such as a substitute for fat and solves some technological problems, probiotic or synbiotic effect, bulking agent or micronutrient premixes, (**Atwaa et al., 2020**).

*Ziziphus spina-christi* (family Rhamnaceae, order of Rosales) tree cultivated in the East and South areas of Asia and the Middle East region (**Yossef et a.l, 2011**). Generally, in Arabic, the fruits have been given the same tree name (**Saied et al., 2008**). In most Arabic countries such as in Saudi Arabia and Egypt, *Ziziphus spina-christi* tree was commonly named Nabaq (related to the Lote-trees of the Quran) while the fruit was called Nabka (**Michel, 2011 and Saaty, 2019**). The plant has been utilized in traditional medicine in Middle East, mainly the fruits, seeds, leaves, roots and bark (**Asgarpanah and Haghghat, 2012**). The fruit of *Ziziphus spina-christi* consider a rich source for important dietary components (**Hamza et al., 2015 and Temerk et al., 2017**)The fruit also contains high amount of vitamin C which is a considerably larger amount compared to those present in strawberry, orange, and grape (38 mg).

The fruits has rich amount of polyphenols (**Alhakmani et al., 2014, Hashem and Abd El-Lahot, 2021**). Phenolic compounds are important natural antioxidants that possess scavenging activity against free radicals (**Weidner et al., 2012**). Sider fruit is used to heal several ailments such as liver complaints, urinary issues, digestive syndromes, weakness, obesity, diabetes, skin infection, appetite loss,

fever, bronchitis, pharyngitis, anemia, insomnia and diarrhea (**Al-Ghamdi et al., 2019**).

The aim of this study to determine of physicochemical, antioxidant activity and total phenolic content of Nabaq fruit and evaluation the effect of added dried Nabaq fruit on physicochemical, rheological, and sensory properties of low fat yoghurt and to investigate the role of yoghurt fortified with dried Nabaq fruit as hypoglycemia agents.

## ***Materials and Methods***

### **Materials**

Three kg of the fresh Nabaq fruits (*Ziziphus spina- christi*), were purchased from the local market, Sharkia, Egypt. The fresh Nabaq fruits (Fig. a) were cleaned with tap water and carefully separated into pulps and seeds using astainless-steel knife. Fruit pulp was dehydrated at 45°C for 12 h in a thermostatically controlled hot air oven. The dehydrated pulp was ground using a milling machine (Culatti Hammer Mill DCFH 48, Germany), then packed in air-tight Kilner jar and stored in a refrigerator (4°C) until used. Fresh buffalo's milk (3% fat) was obtained from Dairy Technology Unit, Food Science Department, Faculty of Agriculture, and Zigzag University, Egypt. Starter Cultures: *Streptococcus salivarius subsp. thermophilus* EMCC104 and *Lactobacillus delbruekii subsp. bulgaricus* EMCC1102 Were obtained from the Microbiological Resources Center (MIRCEN), Faculty of Agric. Aim Shams Univ.,

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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Egypt. DPPH reagent (1, 1- diphenyl - 2 -picrylhydrazyl) and all chemicals used were of analytical grade and purchased from Sigma Company, Germany. The kits utilized for the biochemical analyses were purchased from Gamma Trade Company, Cairo Egypt. The basal pellet diet was obtained from the central animal house of the National Research Center, Dokki. Water was available ad libitum. Male rats weighing 180-230 g were obtained from the Agricultural Reached Center, Giza, Egypt.

**Methods**

**Manufacture of yoghurt:**

Milk containing 3% fat was used in the preparation of yoghurt and served as a control (C). Low fat buffalo's milk (1% fat) was divided into 4 portions. The first portion was left without additive as a control (C1), dried Nabaq fruit was added to the other three portions at the rate of 2 and 4 % (T1 and, T2). The fortified milk bases were homogenized and heated to 90 °C for 15 min., then, cooled to 42 ± 1 °C, inoculated with 2% of yoghurt starter cultures, filled in plastic cups and incubated at 42 °C until a uniform coagulation was obtained. The yoghurt samples from all treatments were stored at 6 ± 1 °C and analyzed at fresh time (*Kebery and Hussein, 1999*).

**Chemical analysis:**

Total solids, fat, total protein (TP) contents, titratable acidity and dietary fiber of yoghurt samples were determined according to *AOAC, (2007)*. The changes in pH in the yoghurt samples during



storage were measured using a laboratory pH meter with glass electrode (HANNA, Instrument, Portugal). Carbohydrate, crude fiber ,ash , calcium, potassium and sodium contents were determined according to **AOAC, (2007)**.while iron and zinc content were determined according to **Page et al., (1992)**.

**Rheological measurements:**

The viscosity and released whey from yoghurt samples was measured according to the method of **Aryana, (2003)**.The quantity of whey collected from every sample in graduated cylinder after 2 h of drainage at 20 °C was used as index of syneresis. Viscosity of nonfat yoghurt samples was determined using Rotational Viscometer Type Lab. Line Model 5437. Results expressed as Centipois (CPS).

**Sensory evaluation:**

The sensory properties of yoghurt samples were assessed by 10 panel members of the Dairy Sci., Dep., Fac. Agric., Zagazig, Univ. They evaluated 20 g portions of each yoghurt sample and used a quality rating score card for evaluation of color & appearance (9 points), flavor (9 points), body& texture (9 points), consistency (9 points) and overall acceptability as described by **Nelson and Trout (1981)**.

**Determination of ascorbic acid content:**

Ascorbic acid was determined using 2, 6 dichlorophenol indophenol dye as described in **AOAC (2007)**.

**Determination of total phenolic content:**

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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The concentration of total phenols in *B. vulgaris* leaves were measured by a UV spectrophotometer (Jenway-UV-VIS Spectrophotometer), based on a colorimetric oxidation/ reduction reaction, as described by **Laličić-Petronijević et al. (2016)**.

**Determination of total flavonoids content:**

Total flavonoids content in the aqueous and ethanolic extracts was determined spectrophotometrically by the method of **Zarina and Tan (2013)**.

**Radical scavenging activity (Scavenging DPPH):**

The antioxidant activity was evaluated by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay according to **Brand Williams et al,(1995)**. The scavenging activity percentage (AOA %) was determined according to **Mensor et al,(2001)** as follows:

$$\text{AOA}(\%) = 1 - \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

**Experimental design of biological study:**

Forty-eight male adult Wistar rats weighing 180-230 g were collected from Agricultural Reached Center, Giza, Egypt. All animals kept under controlled conditions of light (12 h of light and 12 h of darkness) with the ambient temperature of 22±2°C and relative humidity of 40 - 60% and free access to water and food in the Animals' Room. All animals were allowed free access to standard

diet. The normal diet composition was as follows: Casein (20%), sucrose (50%), Corn Starch (15%), powdered cellulose (5%), corn oil (5%), mineral mix (3.5%), vitamin mix (1%), DL- Methionine (0.3%), Choline bitartrate (0.2%) according to AIN-93 guidelines (**Reeves et al., 1993**).

After acclimation on a basal diet for seven days Albino rats were classified into two main sections, where the first one (n= 8) received only the standard diet and served as the normal control group while the second one (hyperglycemia rats, n= 32) was subjected to 60 mg/Kg BW Streptozotocin (STZ) intraperitoneally injection. After 24-48h, rats that showing fasting blood glucose more than 200 mg dl<sup>-1</sup> was considered diabetic rats. Diabetic rats were divided into four groups (n = 8 each) .The first did not receive any treatment and served as the hyperglycemia positive control. The second, third and four ones received 10% low fat yoghurt, 10% low fat yoghurt fortified with 4% dried Nabaq fruit and Meglitinides (0.6 mg/kg body mass) respectively.

The body weight of rats was measured at the beginning of experimental period and after 7 days intervals. At the end of the experiment and after an overnight fasting (10 hr), rats were killed, blood samples were collected and centrifuged at 3000 rpm to obtain the blood serum which will store at (-20°C) for biochemical analysis.

#### **Biochemical assays for lipids:**

Insulin was determined in human blood samples according to the method of (**Thomas, et al., 2014**). Blood glucose level was

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

---

determined according to the method of Clinical Methods (**Trinder, 1969**). Total cholesterol, was determined according to the method of Enzymatic Colorimeter, **Deweerd and Later (2009)**. Total lipids and triglycerides were determined according to the method of **Devi and Sharma, (2004)**.

The LDL was calculated using Friedewald formula (**Friedewald et al., 1972**) as following:

$$LDL\text{-cholesterol} = Total\ cholesterol - (HDL\text{-cholesterol}) - (Triglycerides/5)$$

**Determination of liver function:**

Alanine amino transferase (ALT), aspartate amino transferase (AST) enzymes were measured according to the methods described by **Bergmeyer and Harder, (1986)**, Total bilirubin and total protein were determined according to the methods described by **Tiertz, (1976)**, **Henry, (1974)**, **Moss, (1982)**, **Gowanlock et al.,(1988)** and **Doumas, et al ., (1973)** respectively.

**Determination of kidney functions:**

Urea, uric acid and creatinine were determined according to the method of **While, et al., (1970)**, **Henry, (1974)**, and **Houot, (1985)**, respectively.

**Determination of antioxidant status:**

Total antioxidant capacity (TAC) and Malonaldehyde (MDA) were determined in according to the methods described by **Hu, (1994)**, **Aebi, (1974)**, and **Jentsch, et al., (1996)**, respectively.

**Histopathological examination:**

Pancreas, after removal from the rats were processed and stained with hematoxylin and eosin dyes then examined for histopathological changes under the light microscope (**Banchroft et al., 1996**).

**Statistical analysis:**

Finally, statistical data analysis will conducted using the one-way ANOVA test along with the Duncan test that was performed using the SPSS software (Chicago, IL, USA). All data were subjected to statistical analysis according to the procedure reported by **Steel et al. (1997)** and the statistical analysis system program (**SAS, 1996**).

## ***Results and Discussion***

**Chemical composition, minerals content and phytochemical proprieties of dried Nabaq fruit:**

Chemical composition, minerals content and phytochemical proprieties of dried Nabaq fruit is illustrated in Table (1). Moisture, protein, fat, ash and fiber contents of dried Nabaq fruit were (16.94, 4.72, 0.52, 4.01 and 3.12 g/100g, respectively. This result agrees with **Ahmed and Sati (2018)** and **Hashem and Abd El-Lahot (2021)** who found that Moisture, protein, fat, ash and fiber contents of

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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Dried Nabaq fruit were (17.65, 4.58, 0.59, 3.89 and 2.51 g/100g, respectively. Also, data in Table (1) reveal that, the vitamin C, total phenolic and flavonoids content of dried Nabaq fruit were 19.22, 1986.8 and 192.4 mg/100g, respectively. While the radical scavenging activity (RSA %) was 91.60. Similar results were obtained by **Ahmed and Sati (2018)** who found that ascorbic acid content was 8.64 mg/100 g in Nabaq fruit pulp. Also, **Hashem and Abd El-Lahot (2021)** found that the vitamin C, total phenolic and flavonoids content of dried Nabaq fruit were 18.95, 2200.10 and 180.01 mg/100g, respectively.

The same Table (1) shows the minerals content of Dried Nabaq fruit .The results showed that Ca, K, Na Zn, Fe and Zn contents of Dried Nabaq fruit were (134.20, 810.34, 75.80, 12.18. and 15.42 (mg/100 g dry weight basis) respectively. These results are in agreement with the data obtained by **Hashem and Abd El-Lahot (2021)** found that Dried Nabaq fruit containing Ca (146.53 mg/100 g), K (790.75 mg/100 g), Na (80.33 mg/100 g), Fe (10.20 mg/100 g) and Zn (14.91 mg/100 g) also, **El Maaiden et al. (2020)** who exhibited that Ca, K, and Na of Nabaq fruit were 78.37 to 74.80, 203.20 to 179.65, and 33.70 to 22.77 mg/100 g, respectively .

**Chemical composition of low fat yoghurt supplemented with dried Nabaq fruit :**

Results illustrated in Table (2) show that the highest total solids (TS) were observed in full fat yoghurt (3% fat) compared with

reduced fat yoghurt treatments. Supplementation low fat yoghurt with dried Nabaq fruit increased gradually the TS contents with increase the supplementation ratios. On the other said the lowest protein content was observed in control full fat yoghurt (C) compared to low fat treatments. Supplementation low fat yoghurt with dried Nabaq fruit slightly increased protein content with increase the supplementation ratios. Regarding fat content, addition of dried Nabaq fruit to reduced fat milk did not affect the fat content of reduced fat yoghurt treatments. With respect to fiber content supplementation low fat yoghurt with dried Nabaq fruit increased fiber content and these increments were proportional to the supplementation ratio. These results are the line with those reported by *Atwaa et al,(2020)* who found that supplementation of low fat probiotic yoghurt with mango pulp fiber waste increased TS , protein , ash and fiber contents of low fat yoghurt samples.

**Acidity, pH, whey syneresis, viscosity, total phenolic content and radical scavenging of low fat yoghurt supplemented with dried Nabaq fruit :**

Data presented in Table (3) reveal that control low fat yoghurt contained the highest acidity, compared with full fat yoghurt; supplementation of low fat yoghurt with dried Nabaq fruit decreased the acidity. The pH values of the dried Nabaq fruit fortified yoghurt samples took an opposite direction to the acidity values.

The supplementation of low fat yoghurt with dried Nabaq fruit significantly decreased whey separation, compared with control low fat yoghurt .Control low fat yoghurt had the highest whey separation

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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compared to other treatments. Results in Table (3) also show that control low fat yoghurt (C1) was significantly lower viscosity, compared with full fat and supplemented low fat yoghurt. Supplementation of low fat yoghurt with dried Nabaq fruit increased the viscosity of reduced fat yoghurt. Also, data presented in Table 3 show that TPC (mg /100 g) and RSA % of reduced fat yoghurt enriched with dried Nabaq fruit increased by increasing the supplementation ratio.

The current data align with *Dabija et al, (2018)*, who found that addition of inulin, pea, oat and wheat to yoghurt reduced its acidity, whey syneresis and increased viscosity, TPC and RSA% than control yoghurt. Also, *Atwaa et al, (2020)* found that addition of mango pulp fiber waste powder to low fat yoghurt reduced its acidity, whey syneresis and increased viscosity, TPC and RSA% than control yoghurt.

**Organoleptic characteristics of low fat yoghurt supplemented with dried Nabaq fruit:**

From data presented in Table (4), it can be seen that, the lowest organoleptic characteristics scores was observed in control low fat yoghurt (C1), while adding dried Nabaq fruit to low fat yoghurt enhanced its organoleptic properties and this enhancement was proportional to the supplementation ratio, low fat yoghurt enriched with 4% dried Nabaq fruit was similar to the control full fat yoghurt (3% fat). Similar results were obtained by *Al-hamdani et al,*



(2015) and *Atwaa et al, (2020)* who found that supplementation of low fat yoghurt with 2 and 4% of lupine or 3 % mango pulp fiber waste powder showed the positive effect on sensory scores.

**Effect of low fat yoghurt supplemented with dried Nabaq fruit on blood glucose and insulin of rate groups:**

Data presented in Table 5, shows that in STZ-induced diabetic rats the blood glucose levels were significantly increased ( $p<0.001$ ), while insulin levels of STZ-induced diabetic rats were decreased in comparison to their normal levels. Administration low fat yoghurt fortified with 4 % dried Nabaq fruit in diabetic treated groups lead to significant decrease ( $p<0.001$ ) in serum glucose level as compared with untreated STZ-induced diabetic rats this may be due to ability of Nabaq fruit on decreasing glycosylated haemoglobin (HbA1c), which further damages the blood vessels through oxidative stress by preventing oxidative damage caused by glycation reaction in diabetic conditions as a result of decreased blood glucose level and increased insulin secretion(*Bandawane et al.,2020*) .

Also, a significant decrease ( $p<0.05$ ) was observed in serum glucose level in group 5 treated with Meglitinides than the other group. In line with our results, *Michel et al, (2011)* , *Avize et al.(2010)* and *Bencheikh et al ,(2021)* who found that diabetic rats treated with fruit extract of *Zizyphus spina-christi*( Nabaq ) showed increasing in the blood insulin levels and decreasing in the blood sugar levels compared to the untreated diabetic rats group.

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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**Effect of low fat yoghurt supplemented with dried Nabaq fruit on the serum lipid profile of diabetic rate groups:**

From Table 6 , it can be noticed that, group 5 treated with Meglitinides had the lowest total cholesterol (75.50 mg/dl ) compared to another groups, while rat's administration of diet supplemented with low fat yoghurt containing dried Nabaq fruit recorded significant decrease in total cholesterol (79.13 mg/dl) compared to positive and negative control .

Concerning triacylglyceride and LDL rat (+ve) control group had the highest content of triacylglyceride and LDL (154.20 and 114.50 mg/dl ,respectively ) compared to all groups, while rat's treated with Meglitinides and low fat yoghurt containing Dried Nabaq fruit had the lowest recorded significant decrease in triacylglyceride and LDL values compared to positive and negative control . As for HDL content , (+ve) control group had the lowest HDL content (31.40 mg/dl ) compared to others group, while rat's administration of diet supplemented with low fat yoghurt containing dried Nabaq fruit recorded significant increase in HDL content compared to positive and negative control .

There was significant increase ( $P < 0.05$ ) in the levels of serum cholesterol, triacylglyceride and LDL-c and a decrease in level of HDL-c in the positive control when compared to normal fed rats. Elevated level of blood cholesterol especially LDL-c is a known major risk factor for CHD whereas HDL-c is cardio protective (*Pankaj et al.*,

2010). Treatment with low fat yoghurt containing dried Nabaq fruit *significantly* decreased the levels of total cholesterol, triglycerides and LDL-c as compare to the positive control. Positive control causes the oxidative stress which finally increases production of reactive oxygen species.

An increasing scientific literature provides ample direct. Or indirect evidence that overproduction of ROS can induce cellular damage via oxidation of critical cellular components such as membrane lipids, proteins, and DNA. Since the result of the study indicated that *B. vulgaris* leaves extract has beneficial effect on lipid profile we have investigated its mechanism of action this may be due to phenolic compounds as natural antioxidants present in dried Nabaq fruit (**Ahmed and Sati (2018) and Hashem and Abd El-Lahot (2021)**). Similar results were obtained by **Khader et al., (2017) and Bencheikh et al ,(2021)** ,who indicated that the ethanol extraction of Nabaq fruit decreased ( $P<0.05$ ) in the levels of serum cholesterol, triacylglyceride and LDL-c and increased in level of HDL-c in diabetic rats.

**Effect of low fat yoghurt supplemented with dried Nabaq fruit on total antioxidant capacity (TAC) and malondialdehyde (MDA) of diabetic rate:**

Results of antioxidative indices (TAC and MDA) are summarized in Table (7). As for total antioxidant capacity (TAC) and malondialdehyde (MDA), there were significant differences among the groups. Mean value of TAC was  $740\pm 14$   $\mu\text{mol/L}$  for negative control, then decreased significantly compared to positive control

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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( $510 \pm 0.21$   $\mu\text{mol/L}$ ) by enhancing food in the low fat yoghurt containing dried Nabaq fruit. There is a significant increase in diabetic rat groups treated with low fat yoghurt and low fat yoghurt containing dried Nabaq fruit with  $615 \pm 58$  and  $720 \pm 46$   $\mu\text{mol/L}$ , respectively. Group 5 treated with Meglitinides had the highest TAC value with  $770 \pm 35$ .

Concerning MDA, it can be noticed that positive control was  $4.5 \pm 0.8$  which considered the highest mean value of MDA compared to negative control which recorded the lowest value ( $1.33 \pm 0.4$ ). There is a significant decrease in diabetic rat groups treated with low fat yoghurt and low fat yoghurt containing dried Nabaq fruit with  $3.1 \pm 0.2$  and  $2.3 \pm 0.4$   $\mu\text{mol/L}$ , respectively. Group 5 treated with Meglitinides had the lowest MDA value with  $1.75 \pm 0.6$  compared to positive control.

Malondialdehyde has a very devastating process altering the structure and function of cell membranes (**Nair and Nair, 2015**). **Koc (2003)**, demonstrated that the formation and increase of MDA level can lead to oxidative mechanisms, high cytotoxicity and inhibitory actions. MDA acts as a tumor promoter and co-carcinogenic agent. On the other side, the increase of total antioxidant capacity (TAC) is evidence of improved antioxidative status according to **Joshi et al. (2015)**. Similar results were obtained by **Al-Ghamdi et al, (2019)** who indicated that the ethanol extraction of Nabaq fruit decreased in the levels of MDA and increased in levels of TAC in diabetic rats.

**Effect of low fat yoghurt supplemented with dried Nabaq fruit on AST, ALT and ALP parameters in diabetic rate:**

Data illustrated in Table (8) showed that the untreated group (positive control) showed significant increase in AST, ALT and ALP at ( $p < 0.05$ ) in comparing with normal control group. On the other hand the treated group showed significant decrease in AST, ALT and ALP comparing with positive control group.

AST, ALT and ALP parameters of different treated rat groups were reduced respectively as follows (undiabetic rat (negative control) , rat group treated with Meglitinides , rat group treated low fat yoghurt supplemented with dried Nabaq fruit and rat group treated with low fat yoghurt) in comparison to untreated group (positive control).

This decrease in the values of aminotransferase enzymes and the restoration of some vital functions by the hepatocytes can be attributed to the high content of Nabaq fruit of antioxidants, which work to preserve the plasma membrane in hepatocytes and protect it from rupture and the exit of the cytosol loaded with these enzymes.

( *Bencheikh et al ,2021* ) These results were collaborated by *Abubakar et al.(2018)*, who found that Nabaq fruit supplementation effectiveness in decrease in AST ,ALT and ALP comparing with positive control group.

**Effect of low fat yoghurt supplemented with dried Nabaq fruit on creatinin, uric acid and urea parameters in in diabetic rate:**

Data presented in table (9) showed that the untreated group (positive control) showed significant increase in creatinin, uric acid

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

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and urea at ( $p < 0.05$ ) in comparing with normal control group. On the other hand the treated group showed significant decrease in creatinin, Uric acid and Urea comparing with positive control group. Creatinin, Uric acid and Urea of different treated rat groups were reduced respectively as follows (undiabetic rat (negative control) , rat group treated with Meglitinides , rat group treated with low fat yoghurt containing dried Nabaq fruit and rat group treated with 2 low fat yoghurt) in comparison to untreated group (positive control). These results were collaborated by *Khader et al.,(2017)* and *Al-Ghamdi et al ,(2019)* who found that Nabaq fruit supplementation effectiveness in decrease in uric acid, and urea comparing with positive control group.

**Light microscopic histological study:**

**Group 1 (Control group = G1):**

Light microscopic examination of stained sections with H & E from the pancreas of G1 revealed multiple normal pancreatic lobules and thin CT septa. The exocrine parenchyma had numerous pancreatic acini of pyramidal acinar cells, while the islets of Langerhan's appeared as large pale oval areas with a well-defined contour between the exocrine acini. They consisted of multiple small pale  $\beta$  cells and few large acidophilic rounded alpha cells around small blood capillaries.

**Group II (Streptozotocin (STZ) treated diabetic rats):**

Light microscopic examination of stained sections with H & E from the pancreas of G2 rats showed distorted pancreatic lobules and acini separated by edema and contained dilated ducts. The acinar cells had few vacuoles and most of the islets of Langerhan's were reduced in number. (Figure 2).

**Group III: (STZ-treated diabetic rats & 20% low fat yoghurt):**

Light microscopic examination of stained sections with H & E from the pancreas of G3 revealed multiple normal pancreatic lobules and thin CT septa. The exocrine parenchyma had numerous pancreatic acini of pyramidal acinar cells, while the islets of Langerhan's appeared as a well-defined large pale areas between the exocrine acini. (Figure 3).

**Group IV (STZ-treated diabetic rats & 20% low fat yoghurt containing dried Nabaq fruit)**

Light microscopic examination of stained sections with H & E from the pancreas, liver and kidney of G4 revealed multiple normal pancreatic lobules of numerous exocrine pancreatic acini, while the islets of Langerhan's appeared as a well-defined large pale oval areas around small blood capillaries and between the exocrine acini. (Figure 4).

**Group V: (STZ-treated diabetic rats & Meglitinides-treated diabetic rats):**

Light microscopic examination of stained sections with H & E from the pancreas of G5 rats showed distorted pancreatic lobules, acini, and contained dilated ducts. The acinar cells had few vacuoles

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

and most of the islets of Langerhan's were reduced in number. (Figure 5).

### **Conclusion**

Nabaq (*Zizyphus spina-christi* L) fruit showed strong antioxidant capacity and high content of phenolic component. Therefore, dried Nabaq (*Zizyphus spina-christi* L) fruit could be used as natural additives in manufacture of low fat yoghurt to improve its physicochemical, rheological and sensory properties. Also, the observed blood sugar reducing action of the Nabaq (*Zizyphus spina-christi* L) fruit powder indicates the hypoglycemia activity.

**Table (1):**Chemical composition, minerals content and phytochemical proprieties of dried Nabaq fruit

Chemical composition	
Moisture (%)	16.94±0.45
Total protein (%)	4.72±0.18
Fat (%)	0.52±0.03
Ash (%)	4.01±0.12
Fiber (%)	3.12±0.25
Minerals (mg/100 g dry weight basis)	
Calcium (Ca )	134.20±4.3
Potassium (K)	810.34±6. 8
Sodium (Na)	75.80±2.7
Iron (Fe)	12.18±1.02



Zinc ( Zn)	15.42±1.18
Phytochemicals	
Vitamin C (mg/100 g)	19.22±0.40
Total phenolic content(mg/g)	1986,8±8.6
Total flavonoids( mg/ 100 g)	192.4±4.3
Radical scavenging activity (%)	91.60±3.6

**Table 2:**Effect of supplementation low fat yoghurt with dried Nabaq fruit on chemical composition

Components (%)	Treatments			
	C	C <sub>1</sub>	T <sub>1</sub>	T <sub>2</sub>
Total Solids	12.14±0.30 <sup>a</sup>	10.24±0.42 <sup>d</sup>	11.02±0.38 <sup>b</sup>	11.86±0.32 <sup>c</sup>
Fat	3.10±0.14 <sup>a</sup>	1.12±0.07 <sup>b</sup>	1.14±0.05 <sup>b</sup>	1.18±0.04 <sup>b</sup>
Protein	3.60±0.22 <sup>b</sup>	3.94±0.16 <sup>a</sup>	3.98±0.10 <sup>a</sup>	4.02±0.08 <sup>a</sup>
Fiber	-	-	0.05±0.01 <sup>b</sup>	0.08±0.02 <sup>a</sup>

\* Values (means ±SD) with different superscript letters are statistically significantly different ( $P \leq 0.05$ ).

C: Control full fat yoghurt (3% fat)

C<sub>1</sub>: Control low fat yoghurt (1%

fat).

T<sub>1</sub>: Low fat yoghurt containing 2% dried Nabaq fruit

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

T<sub>2</sub>: Low fat yoghurt containing 4% dried Nabaq fruit

**Table 3:**Effect of supplementation low fat yoghurt with dried Nabaq fruit on acidity, pH, whey syneresis, viscosity, total phenolic content and radical scavenging

Parameters	Treatments			
	C	C1	T <sub>1</sub>	T <sub>2</sub>
Acidity %	0.80±0.03 <sup>b</sup>	0.88±0.04 <sup>a</sup>	0.83±0.05 <sup>ab</sup>	0.78±0.03 <sup>c</sup>
pH	4.68±0.05 <sup>b</sup>	4.28±0.06 <sup>d</sup>	4.60±0.03 <sup>c</sup>	4.72±0.05 <sup>a</sup>
syneresis (ml/100gm)	21.00±1.14 <sup>d</sup>	30.00±1.32 <sup>a</sup>	28.00±1.20 <sup>b</sup>	25.00±1.14 <sup>c</sup>
Viscosity (C. P.S.)	5490±8.2 <sup>a</sup>	4280±10.5 <sup>d</sup>	4320±9.20 <sup>c</sup>	4520±10.0 <sup>b</sup>
TPC (mg /100 g)	52.70±2.4 <sup>c</sup>	44.50±2.46 <sup>d</sup>	62.80±1.77 <sup>b</sup>	80.30±1.65 <sup>a</sup>

## Egyptian J. of Nutrition Vol. XXXVI No. 2 (2021)

RSA %	22.14±1.0 <sup>c</sup>	18.86±1.22 <sup>d</sup>	30.44±1.12 <sup>b</sup>	38.70±1.34 <sup>a</sup>
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\* Values (means ±SD) with different superscript letters are statistically significantly different ( $P \leq 0.05$ ).

C: Control full fat yoghurt (3% fat)  
fat).

C<sub>1</sub>: Control low fat yoghurt (1%

T<sub>1</sub>: Low fat yoghurt containing 2% dried Nabaq fruit

T<sub>2</sub>: Low fat yoghurt containing 4% dried Nabaq fruit

**Table (4):** Organoleptic characteristics of low fat yoghurt supplemented with dried Nabaq fruit

Properties	Treatments			
	C	C <sub>1</sub>	T <sub>1</sub>	T <sub>2</sub>
Appearance	9.40±0.20 <sup>a</sup>	7.60±0.24 <sup>d</sup>	7.70±0.22 <sup>c</sup>	7.90±0.14 <sup>b</sup>
Flavour	8.80±0.33 <sup>a</sup>	7.50±0.48 <sup>d</sup>	7.90±0.32 <sup>c</sup>	8.20±0.24 <sup>b</sup>
Texture	8.90±0.28 <sup>a</sup>	6.50±0.36 <sup>d</sup>	6.90±0.30 <sup>c</sup>	7.40±0.26 <sup>b</sup>
Consistency	8.50±0.20 <sup>a</sup>	6.20±0.33 <sup>e</sup>	6.70±0.28 <sup>d</sup>	7.50±0.20 <sup>c</sup>
Overall acceptability	8.90±0.28 <sup>a</sup>	6.40±0.48 <sup>d</sup>	7.20±0.33 <sup>c</sup>	8.10±0.22 <sup>b</sup>

\* Values (means ±SD) with different superscript letters are statistically significantly different ( $P \leq 0.05$ ).

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

C: Control full fat yoghurt (3% fat)

C 1: Control low fat yoghurt (1% fat).

T<sub>1</sub>: Low fat yoghurt containing 2% dried Nabaq fruit

T<sub>2</sub>: Low fat yoghurt containing 4% dried Nabaq fruit

**Table 5:**Effect of low fat yoghurt supplemented with dried Nabaq fruit on blood glucose and insulin of rate groups

Groups	Parameters	
	Blood glucose concentration (mg/dL)	Insulin ( $\mu$ U/ml)
– Group (1):	94.30 $\pm$ 4.80 <sup>c</sup>	17. 20 $\pm$ 0.12 <sup>a</sup>
– Group (2):	260.70 $\pm$ 8.66 <sup>a</sup>	7.40 $\pm$ 0.40 <sup>e</sup>
– Group (3):	102. 90 $\pm$ 5.80 <sup>b</sup>	10.60 $\pm$ 0.32 <sup>d</sup>
– Group (4):	95.80 $\pm$ 5.10 <sup>c</sup>	13.40 $\pm$ 0.22 <sup>c</sup>
– Group (5):	88. 20 $\pm$ 8.30 <sup>d</sup>	14.70 $\pm$ 0.30 <sup>b</sup>

Mean values of six rat's  $\pm$  SD. A, b, c....of the small letters in the same column are significantly different at (p  $\leq$ 0.05)..

## Egyptian J. of Nutrition Vol. XXXVI No. 2 (2021)

Group (1): non-treated non-diabetic rats (negative control).

Group (2) :non-treated diabetic rats (positive control).

Group (3): diabetic rats treated with 10% low fat yoghurt.

Group (4): diabetic rats treated with 10% low fat yoghurt fortified with 4 % dried Nabaq fruit.

Group (5): diabetic rats treated with Meglitinides.

**Table 6:**Effect of low fat yoghurt supplemented with dried Nabaq fruit on lipid profile of diabetic rate groups

Parameter	Total cholesterol (TC) (mg/dl)	Triglycerides (TG) (mg/dl)	HDL-c (mg/dl)	LDLc (mg/dl)
– Group (1):	97.30±3.22 <sup>b</sup>	133.40±8.40 <sup>b</sup>	45.00±2.34 <sup>a</sup>	25.62±1.70 <sup>c</sup>
– Group (2):	114.50±6.54 <sup>a</sup>	154.20±7.20 <sup>a</sup>	31.40±2.50 <sup>d</sup>	52.26±4.52 <sup>a</sup>
– Group (3):	84.44±2.35 <sup>c</sup>	101.12±5.40 <sup>c</sup>	35.64±2.24 <sup>c</sup>	28.58±2.34 <sup>b</sup>
– Group (4):	79.13±2.52 <sup>d</sup>	95.04±4.60 <sup>d</sup>	39.50±2.64 <sup>b</sup>	20.62±3.24 <sup>d</sup>
– Group (5):	75.50±2.38 <sup>e</sup>	89.60±3.20 <sup>e</sup>	43.22±2.40 <sup>a</sup>	14.36±1.40 <sup>e</sup>

Mean values of six rat's ± SD. A, b, c....of the small letters in the same column are significantly different at (p ≤0.05).

Group (1): non-treated non-diabetic rats (negative control).

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

Group (2) :non-treated diabetic rats (positive control).

Group (3): diabetic rats treated with 10% low fat yoghurt.

Group (4): diabetic rats treated with 10% low fat yoghurt fortified with 4 % dried Nabaq fruit.

Group (5): diabetic rats treated with Meglitinides.

**Table 7:**Effect of low fat yoghurt supplemented with dried Nabaq fruit on total antioxidant capacity (TAC) and malondialdehyde (MDA) of diabetic rats

Parameter Groups	Malondialdehyde (MDA) ( $\mu\text{mol/L}$ )	Total antioxidant (FRAP) ( $\mu\text{mol/L}$ )
Group (1):	$1.33 \pm 0.4^e$	$740 \pm 24^b$
Group (2):	$4.5 \pm 0.8^a$	$510 \pm 21^d$
Group (3):	$3.1 \pm 0.2^b$	$615 \pm 58^c$
Group (4):	$2.3 \pm 0.4^c$	$720 \pm 46^b^c$
Group (5):	$1.75 \pm 0.6^d$	$770 \pm 35^a$

## Egyptian J. of Nutrition Vol. XXXVI No. 2 (2021)

Mean values of six rat's  $\pm$  SD. A, b, c....of the small letters in the same column are significantly different at ( $p \leq 0.05$ ).

Group (1): non-treated non-diabetic rats (negative control).

Group (2) :non-treated diabetic rats (positive control).

Group (3): diabetic rats treated with 10% low fat yoghurt.

Group (4): diabetic rats treated with 10% low fat yoghurt fortified with 4 % dried Nabaq fruit.

Group (5): diabetic rats treated with Meglitinides.

**Table 8:**Effect of low fat yoghurt supplemented with dried Nabaq fruit on AST, ALT and ALP parameters in diabetic rate

Parameter Groups	Aspartate aminotransferase (AST U/L )	Alanine aminotransferase (ALT U/L )	Alkaline phosphatase (ALP U/L )
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Group (1):	61.30 $\pm$ 4.16 <sup>d</sup>	22.02 $\pm$ 1.248 <sup>e</sup>	141.80 $\pm$ 6.04 <sup>d</sup>
Group (2):	86.06 $\pm$ 3.40 <sup>a</sup>	36.60 $\pm$ 3.12 <sup>a</sup>	169.54 $\pm$ 5.24 <sup>a</sup>
Group (3):	80.88 $\pm$ 2.12 <sup>b</sup>	34.54 $\pm$ 1.48 <sup>b</sup>	161.30 $\pm$ 5.70 <sup>b</sup>
Group (4):	75.04 $\pm$ 3.84 <sup>c</sup>	32.62 $\pm$ 3.28 <sup>c</sup>	155.56 $\pm$ 6.02 <sup>c</sup>
Group (5):	73.50 $\pm$ 3.90 <sup>c</sup>	30.70 $\pm$ 2.15 <sup>d</sup>	152.26 $\pm$ 6.25 <sup>c</sup>

**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**

Mean values of six rat's  $\pm$  SD. A, b, c....of the small letters in the same column are significantly different at ( $p \leq 0.05$ ).

Group (1): non-treated non-diabetic rats (negative control).

Group (2) :non-treated diabetic rats (positive control).

Group (3): diabetic rats treated with 10% low fat yoghurt.

Group (4): diabetic rats treated with 10% low fat yoghurt fortified with 4 % dried Nabaq fruit.

Group (5): diabetic rats treated with Meglitinides

**Table 9:**Effect of low fat yoghurt supplemented with dried Nabaq fruit on creatinin, uric acid and urea parameters in diabetic rate

Parameter Groups	Creatinin Mg/dl	Uric acid Mg/dl	Urea Mg/dl
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Group (1):	0.86 $\pm$ 0.06 <sup>d</sup>	2.38 $\pm$ 0.16 <sup>d</sup>	26.52 $\pm$ 1.05 <sup>d</sup>
Group (2):	1.75 $\pm$ 0.14 <sup>a</sup>	4.72 $\pm$ 0.14 <sup>a</sup>	44.08 $\pm$ 3.22 <sup>a</sup>
Group (3):	1.24 $\pm$ 0.10 <sup>b</sup>	3.50 $\pm$ 0.28 <sup>b</sup>	32.04 $\pm$ 2.12 <sup>b</sup>
Group (4):	1.10 $\pm$ 0.08 <sup>bc</sup>	3.22 $\pm$ 0.34 <sup>bc</sup>	29.40 $\pm$ 2.18 <sup>c</sup>
Group (5):	0.96 $\pm$ 0.04 <sup>c</sup>	3.04 $\pm$ 0.26 <sup>c</sup>	27.10 $\pm$ 2.24 <sup>d</sup>



## Egyptian J. of Nutrition Vol. XXXVI No. 2 (2021)

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Mean values of six rat's  $\pm$  SD. A, b, c....of the small letters in the same column are significantly different at ( $p \leq 0.05$ ).

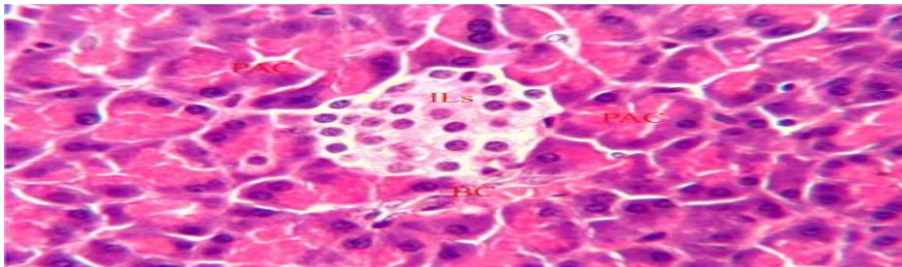
Group (1): non-treated non-diabetic rats (negative control).

Group (2) :non-treated diabetic rats (positive control).

Group (3): diabetic rats treated with 10% low fat yoghurt.

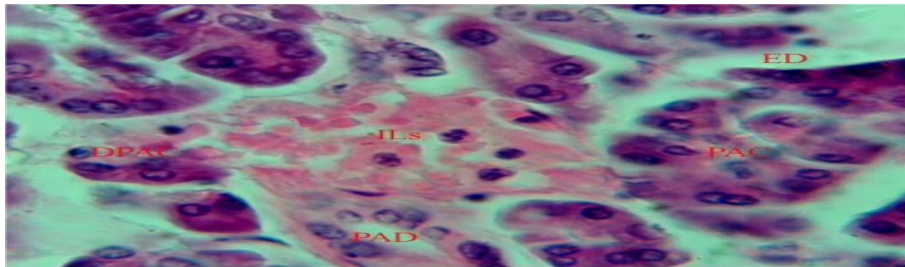
Group (4): diabetic rats treated with 10% low fat yoghurt fortified with 4 % dried Nabaq fruit.

Group (5): diabetic rats treated with Meglitinides

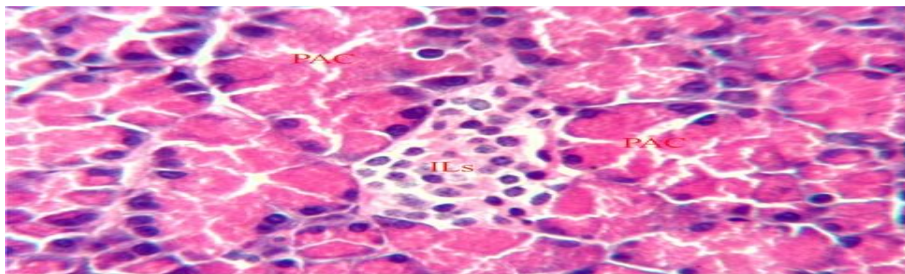


**Figure 1:** Photomicrographs of sections from the pancreatic tissues of G1 adult male albino rats. H & E x640.

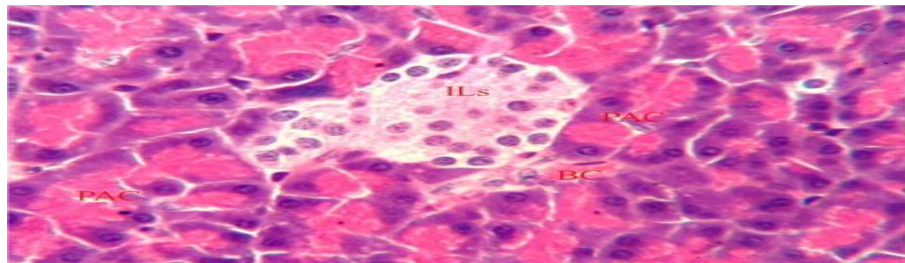
**Atwaa E.H. , Hanan A. Radwan, Hanan S. Shalaby  
and Ghada M. El-Araby**



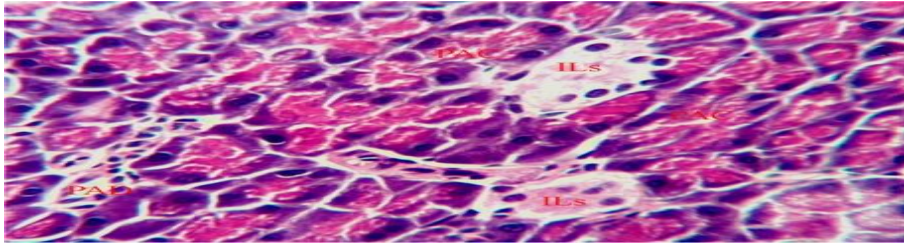
**Figure 2:** Photomicrographs of sections from the pancreatic of G2 adult male albino rats. H & E x640.



**Figure3:** Photomicrographs of sections from the pancreatic of G4 adult male albino rats. H & E x640



**Figure 4:** Photomicrographs of sections from the pancreatic of G5 adult male albino rats. H & E x640.



**Figure 5:** Photomicrographs of sections from the pancreatic of G5 adult male albino rats. .H & E x640

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الفعل العلاجي للزبادي المنخفض الدهن المدعم بمسحوق فاكهة السدر على  
ارتفاع سكر الدم في الفئران المصابة بداء السكري المستحث بفعل  
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### الملخص

تحتوي فاكهة السدر على تركيبة غنية من المكونات النشطة بيولوجيًا مثل الفينول والفلافونويد والألياف الغذائية والمعادن والفيتامينات. أجريت الدراسة لتقييم تأثير التدعيم بمسحوق ثمار السدر على الخواص الفيزيوكيميائية والريولوجية والحسية للزبادي قليل الدسم (١٪ دهن). حيث تمت إضافة مسحوق ثمار السدر إلى اللبن الزبادي قليل الدسم بنسب ٢ و ٤٪ وأظهرت النتائج زيادة تدريجية في الأس الهيدروجيني والألياف الغذائية واللزوجة والمحتوى الفينولي وقيم النشاط المضاد للأكسدة في الزبادي قليل الدسم مع زيادة نسبة مسحوق ثمار السدر. من ناحية أخرى ، انخفضت قيم الحموضة ومعدل انفصال الشرش بزيادة نسبة مسحوق ثمار السدر. واعطي الزبادي قليل الدسم المحتوي على ٣٪ من مسحوق ثمار السدر قيم أعلى من حيث الخصائص الفيزيوكيميائية و الحسية والريولوجية عن المعاملات الأخرى. تم تقييم الزبادي قليل الدسم المدعم بنسبة ٣٪ من مسحوق ثمار السدر كعامل خافض لسكر الدم الناجم عن الأستربتوزوتوسين في الفئران المصابة بداء السكري. حيث تم تقسيم أربعين من ذكور الفئران إلى ٥ مجموعات على النحو التالي: المجموعة (١) الفئران غير المعاملة وغير المصابة بالسكري (المقارنة السلبية). المجموعة (٢) الفئران المصابة بداء السكري (تناولت Streptozotocin (STZ ، 65مجم / كجم من وزن الجسم) (التحكم الإيجابي) المجموعة (٣) الفئران المصابة بداء السكري والمعاملة بـ ٢٠٪ زبادي قليل الدسم بدون أي إضافات. المجموعة الرابعة: فئران مصابة بمرض السكر والمعاملة بـ ٢٠٪ زبادي قليل الدسم مدعم بنسبة ٤٪ مسحوق فاكهة السدر. المجموعة (٥) الفئران المصابة بداء السكري المعاملة بالـ Meglitinides كدواء معترف به للسكري: أظهرت النتائج ان معامل الفئران المصابة بداء السكري بالزبادي قليل الدسم المحتوي على مسحوق فاكهة السدر انخفضاً معنوياً ( $p < 0.001$ ) في مستويات السكر في الدم ، المالونديالدهيد (MDA) ، البروتين الدهني

منخفض الكثافة (LDL) ، الكوليسترول (CL) ، الدهون الثلاثية (TG) ، AST ، ALT ، ALP ، الكرياتينين ، حمض اليوريك واليوريا وزيادة ( $p < 0.001$ ) الأنسولين والبروتين الدهني عالي الكثافة (HDL) والقدرة الإجمالية لمضادات الأكسدة (TAC) مقارنةً بفئران المقارنة الايجابية لمرض السكري . وهكذا أوضحت الدراسة أن مسحوق ثمار السدر يمكن استخدامه كمصدر للمركبات النشطة بيولوجيا في صناعة الزيادي قليل الدسم مما يعزز قيمته الغذائية وخصائصه الريولوجية والحسية ويجعل له تأثير خافض لسكر في الدم.