

Biological Assay of Diabetic Children Cookies Prepared From Plant Sources

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Abstract

This work was aimed to prepare high nutritional value cookies for diabetic children and as a blood sugar lowering materials. Four cookies for diabetic children of high nutritive value were prepared using some plant seeds (black rice, lupine and germinated fenugreek seeds) and leaves (mulberry and olive leaves). The effect of sugar substitution on biological properties of prepared snacks using different levels of natural and artificial sweeteners { stevia, high fructose corn syrup (HFCS) } and olive oil were carried out.

Rats were randomly assigned to (7 groups) four rats each, extending feeding albino rats for six weeks on any investigated cookies which contained plant sources such as seeds and leaves caused a continuous decrease in glucose levels of rats. Cookies 2 which was benefit for reducing glucose blood levels, where it was (146.50 mg/dl) at the end of the six weeks compared with control (-) and (+) cookies. It was noticed that rats group fed on control (-) was the highest body weight gain (35.75) with being significantly different with other rats groups fed on cookies compared with control (+) showed the lowest BWG (-17.67). For food daily intake, it was clearly that it increased in all treated groups compared with positive control group (17.68) comparing with negative control (17.75). Highest FER was (3.18) in cookies 2 rat groups followed by (3.05) for control cookies rat groups comparing with control (-) which recorded (2.02).

Control (-) had low content of cholesterol (121.21 mg/dl) followed by cookies 3 rat groups as (123.82). The highest HDL-C was found to be in control (-) followed by cookies 3 rat groups as (53.80 mg/dl) and (45.87 mg/dl), respectively. Rats groups fed on cookies 3 were the lowest LDL-C content (52.19 mg/dl) compared with control (+) as (66.96 mg/dl). The highest GOT content was (69.50) found in rat groups fed on cookies 4. Feeding rat groups on diabetic children diets effected positively on high density lipoprotein cholesterol (HDL-C) and negatively on cholesterol, low density lipoprotein cholesterol (LDL-C) and triglyceride as well as reducing glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT).

Keywords: Biological Assay, Diabetic Children, Cookies, Plant Sources, (GOT) and (GPT).

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide that is caused by an absolute or relative lack of insulin and or reduce insulin activity (**Jemaiet *al.*, 2009**). As a very common chronic diseases, diabetes are becoming the third "killer" of man health along with cancer, cardiovascular and cerebrovascular diseases because of its high prevalence, mobility and mortality. As reported by **WHO (2000)**, in Egypt there are about 5 million diabetics. The prevalence of diabetes mellitus in Egypt and all over the world caused many problems. One of them is the complications on the body organs besides the potential side effects of insulin injection or oral hypoglycemic agents. The other one is the high cost of drugs.

In spite of the fact that insulin has become one of the most important therapeutic agents know to medicine, research workers again have been making efforts to find insulin substitutes from plant sources for the treatment of diabetes. Many of them have depressed areas where insulin is not readily (**Sanehezet *al.*, 1994**). The discovery of a great number of sweeteners during the last decade has triggered the development of new sugar-free products, particularly for diabetics, people on special diets and/or for the obese (**Ozdemir and Sadikoglu, 1998**). Control of diabetes by spices and other natural products is becoming popular and is more appropriate and economical for use developing countries (**Safdaret *al.*, 2006**).

Black rice (*Oryza sativa L. indica*), a special cultivar of rice which contains a much higher content of anthocyanins in the aleurone layer than white rice, has been regarded as a food and widely consumed as a health-promoting food in China and other Eastern Asia countries for thousands of years (**Wang *et al.*, 2007**). Black rice contains anthocyanin pigments, such as cyaniding and peonidin glycosides, in the bran layer. Anthocyanin is known to have physiological functions, as it has antioxidative activities, contributes to the prevention of artherosclerosis, and is a type of functional food (**Koguchiet *al.*, 2009**).

Lupine seed flour can be used in production of different products. It can be added to pasta, crisps, bread and meat products to increase nutritional value, aroma as well as modify the texture of the end products. Moreover, protein isolate produced from lupine seeds can be utilized for milk and meat imitation products. In the Middle East, lupine seeds are consumed as a snack after they soaked in water (**Tizazu and Emire, 2010**).

Lupine enriched foods have the potential to beneficially influence glycaemic control (**Magniet *et al.*, 2004**). Improve blood lipids (**Nowicka *et al.*, 2006** and **Spielman *et al.*, 2007**). Fenugreek, a member of the genus *Trigonella*, and has the Arabic name "Helba", has been shown to act as hypoglycemic and hypocholesterol-olemic agent in both animal and human studies (**Madar and Stark, 2002**).

Although very little information is available on the nutrient composition of mulberry leaves, reports indicate that mulberry leaves contain appreciable amounts of various nutrients, especially protein (**Sreekumar *et al.*, 1994**). Leaves also contain carbohydrates, oils, minerals and vitamins, especially ascorbic acid. The mulberry leaf powder has been found to be effective in treatment of obese diabetic patients and hypertensive patients (**Suryanarayana, 2002**).

Olive leaves are considered as a cheap raw material which can be used as a useful source of high-added value products. The main phenolic compounds in olive leaves are the glycosylated forms of oleuropein and ligstroside. The main active component in olive leaf extract is oleuropein a natural product of the secoiridoid group (**Jema *et al.*, 2009**).

Olive oil is the principal source of added lipid in the Mediterranean diets. Virgin olive oil is a natural product that is low in saturated fatty acids and high in monounsaturated fatty acids. In addition, it contains a balanced amount of essential fatty acids (linoleic and linolenic acids) that are adequately protected by natural antioxidants (**Vasilopoulou *et al.*, 2005**).

Stevia leaves are delicious food, enhancing their natural flavour and no calorie sweeteners. Then leaves contain mixture different glycosides derived from tetra-cylic- diterpenesteriol (Steviolbioside, steviosiderebaudiosides A, B, C, D, E and dulcosides). This natural products taste intensity sweet 250-300 times as sweet as sucrose (**Richman *et al.*, 1999**). Stevia is rich nutrients, containing substantial amount of protein, calcium, phosphorus and other important nutrients (**Kinghorn and Soejarto, 1985** and **Tsanava *et al.*, 1989**).

Stevia leaves are important source of natural sugar substitute due to its low caloric value and taste, which is very similar to sucrose. The extracted sweeteners are commonly used as nutritive and possess a high-intensity sweet that could be used in beverages, foods and medicines (**Badr and El-sanat, 2008**). High fructose corn syrup (HFCS) could be used to replace sucrose in large or small preparations of all food products (**Inglett, 1981**).

"Cookies" is chemically leavened product, also known as "biscuit". Generally the term biscuit is used in the European countries and cookies in the

USA. Cookies are ideal for nutrient availability, palatability, compactness and convenience. They differ from other baked products like bread and cake because of having low moisture content, comparatively free from microbial spoilage and long shelf life of the product (**Sharif et al., 2009**).

The present study was directed to prepare high nutritional value cookies for diabetic children and as a blood sugar lowering materials. This work was investigated also the effect of sugar substitution on biological properties of prepared cookies. Replacement sucrose by different levels of natural and artificial sweetener such as stevia and high fructose corn syrup (HFCS) was happened in the preparation of diabetic children cookies.

Materials and Methods

The raw materials which used throw out this study were: Black rice (*Oryza sativa L.indica*) was obtained from the Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt. Sweet White Lupine seeds (*Lupinus albus L.*) and Fenugreek seeds (*Trigonella foenum-graecum L.*) were obtained from the local market at Kafr El-Sheikh, Egypt. Mulberry leaves (*Morus alba*) and Olive leaves (*Olea europaea*) were obtained from sewa and Kafr El-Sheikh, Egypt.

Leaves were dried in an oven air 40-60 °C for 72 hours. For **sweeteners: Sucrose** (sugar) was obtained from the local market at Kafr El-Sheikh. Stevia leaves (*Stevia rebaudiana*) were obtained from Sugar Crops Research Institute, Giza, Egypt. High fructose corn syrup 55% fructose (**HFCS**) was obtained from National Company of Corn Products- El- Asher of Ramadan City. Olive oil was obtained from Matroh and butter was obtained from the local market at Kafr El-Sheikh, Egypt.

All seeds were cleaned from impurities, broken seeds, dust and other foreign matters. Black rice seeds were husked by dehulling machine. Lupine seeds were cleaned, separated from foreign matters, soaked in water (1:5 w/v) for 12 hours and the water was changed three times during the soaking period. The seeds were dried in a hot air oven 40-60 °C for 96 hours, milled and kept in polyethylene bags until used. Olive and mulberry leaves were cleaned thoroughly by washing. Then, dried leaves were kept in polyethylene bags until used. Brown and yellow stevia leaves were removed from plants then, washed and spread on trays (60×60cm), dried in air oven dryer at 65-70 °C for 10-12 hours (until samples reached to constant weight). Then leaves were weighted and ground in a laboratory mill to obtain stevia powder (**Badr and El-Sanat, 2008**).

After cleaning fenugreek seeds were speredated in 1 cm thick layer between wet cotton clothes avoiding the accumulation seeds and were sprayed with water twice daily, seeds were germinated at room temperature (25 ± 2 °C) up to three days (**El-Bagoury, 2005**). Oven air (60-70 °C) was used for 48 hours to dry the lupine, germinated and toasted fenugreek seeds (**Sidkeyet al., 1993**), then all plants were grounded in an electric mill and sieved at in 120 meshes to produce a fine powder. All samples were kept in polyethylene bags at 5 °C until blended.

Preparation of diabetic children cookies using plant seeds and leaves

Four formulas of cookies were prepared using plant sources, seeds (black rice, lupine and germinated fenugreek) and leaves (mulberry and olive leaves). Wheat flour was substituted with these formulas 10, 20 and 30 %. These formulas were used to make diabetic children cookies using natural sweeteners stevia, high fructose corn syrup. Fats used in these diets were butter and olive oil.

Table (A): Materials used for preparation control cookies

Ingredients	Cookies
Flour (mixtures + wheat)	100
Sugar	25
Corn oil	15
Baking powder	2
Water	25
Vanilla	0.03

Table (A) show the content of control cookies made from 100% wheat flour. The dry ingredients were mixed thoroughly for one minute by hand. Cookie was prepared by adding 100 gm wheat flour (72% extraction), 25 gm sugar, 15 ml corn oil, 2 gm baking powder, 25 ml water and 0.03 gm vanilla. Cookies were formed by biscuits machine and baked in an oven at 135 °C for 45 minutes (**Eneche 1999**).

Table (B): The composition of diabetic children cookies

Diabetic Cookies	Wheat flour %	Percentage of plant additive	Sweeteners	Fats
Control **	100	-----	Sucrose	Corn oil
Cookies 1	90	10 (5% Black rice + 5% Lupine)	Stevia powder	Butter
Cookies	90	10 (5% Lupine + 5% Germinated	HFCS *	Olive

2		fenugreek)		oil
Cookies 3	90	10 (5% Lupine + 5% Toasted fenugreek)	HFCS *	Olive oil
Cookies 4	90	10 (5% Lupine + 5% Toasted fenugreek)	Stevia powder	Butter

* HFCS (High Fructose Corn Syrup).

** Control cookie was prepared from 100% wheat flour.

Cookies:

Cookie was prepared by adding 100 gm wheat flour (72% extraction), 25 gm sugar, 15 ml corn oil, 2 gm baking powder, 25 ml water and 0.03 gm vanilla (Eneche 1999). Cookies were formed by biscuits machine and baked in an oven at 135 °C for 45 minutes.

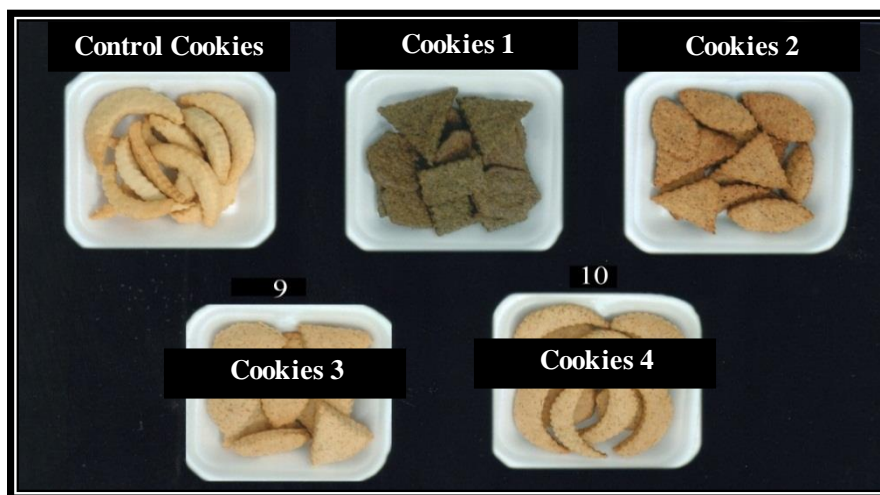


Fig (1): Prepared cookies.

Control cookies: 100 % wheat flour.

Cookies (1): 90 % wheat flour, 10 % (black rice and lupine) used stevia and butter.

Cookies (2): 90 % wheat flour, 10 % (lupine and germinated fenugreek) used HFCS and olive oil.

Cookies (3): 90 % wheat flour, 10 % (lupine and toasted fenugreek) used HFCS and olive oil.

Cookies (4): 90 % wheat flour, 10 % (lupine and toasted fenugreek) used stevia and butter.

Biological evaluation of prepared snacks

Animals

White male albino rats Sprague Dawley (20 rats) weighting 100 gm, on month old were employed in this study. Rats were obtained from experimental animal house of Food Technology Research Institute, Agric. Res. Center, Giza, Egypt. Upon arrival, they were randomly assigned to (7 groups) four rats each. Each animal was individually housed in a wire bottomed, stainless steel cage under the normal condition. The animals were weighted weekly except during the first week, which weighted every day. The experimental animals fed on basal diet for one week to acclimate them to our facility and basal diet. After acclimation, rats were fed on different diets as shown and given in the last classification for rat groups.

Induction of diabetes

Blood glucose was recorded for all rats. Diabetic rats were produced by a single dose of alloxan (150 mg/kg) after 12 hours fasting. After two days of the injection with alloxan, blood glucose concentration was determined. The normal blood glucose level of rats was ranged from 50 to 135 mg/dl (**Arun and Nalini, 2002**). Animals having blood glucose concentration over 190 mg/dl already considered diabetic.

Experimental design and animal groups

This experiment was designated to study the effect of some plant seeds (black rice, lupine and germinated fenugreek) and leaves (mulberry leaves and olive leaves) on diabetic cookies for children using natural sweetener such as stevia and artificial sweetener such as high fructose corn syrup (HFCS) comparing with sucrose. Fats used in these diets were butter and olive oil, for studying its effect on body weight , food intake , also on blood glucose , as well as different serum total cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride, low density lipoprotein (LDL) cholesterol in rats and (GPT) and (GOT). Diabetic and normal rats were fed for 6 weeks. The rats were divided as fellow:

G1: Negative control (normal rats), fed on basal diet.

G2: Positive control (diabetic rats), fed on basal diet.

G3: Rats fed on control cookies.

G4: Rats fed on cookies 10 % (black rice and lupine) used stevia and butter.

G5: Rats fed on cookies 10 % (lupine and germinated fenugreek) used HFCS and olive oil.

G6: Rats fed on cookies 10 % (lupine and toasted fenugreek) used HFCS and olive oil.

G7: Rats fed on cookies 10 % (lupine and toasted fenugreek) used stevia and butter.

Rats were weekly weighted, and food intake, body weight gain (BWG %) and food efficiency ratios (FER) were calculated at the end of the experiment according to (Chapman *et al.*, 1959) using the following formula:

$$\text{BWG \%} = (\text{Final Weight} - \text{Initial} / \text{Initial Weight}) \times 100.$$

$$\text{FER} = [\text{Gain in body (g/day)} / \text{Food intake (g/ day)}] \times 100].$$

$$\text{Food Intake} = \text{Rat body weight} \times (10/100).$$

Basal diet

The basal diet in the experiment consisted of casein (10 %), corn oil (10 %), vitamin mixture (1 %), salt mixture (4 %), choline chloride (0.2 %), methionine (0.3 %), cellulose (5 %), and the remainder is corn starch (69.5 %), according to Campbell (1963).

Determination of rats blood glucose

Blood glucose was measured according to the method described by Allesen *et al.*, (1999) using blood glucose meter (Gluco star 2). A drop of blood was taken from tail of the rat, placed on a test strip and blood glucose was measured immediately with a blood glucose meter (Youssef *et al.*, 2007).

Blood sampling

In all mentioned groups, blood samples were taken from rats at the end of the experiment, the blood samples were collected after 12 hours fasting, and rats weighed and scarified with a knife, blood of rats put into dry clean centrifuge tubes and left to clot. The blood was centrifuged for 10 minutes at 3500 rpm to separate. The serum, which was carefully aspirated and transferred into clean quite plastic tubes and kept frozen at -18°C until biochemical analysis (El-Khamissy, 2005).

Determination of total cholesterol, HDL cholesterol and triglycerides

The concentration of total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides in the serum were determined without extraction by using enzymatic colorimetric methods with commercially available kit#276-64909 ,high density lipoprotein kit#278-67409 and triglyceride, kit#274-69807; wake chemical, Osaka, Japan. Kim and Shin (1998) procedures were employed to perform the previous mentioned determinations.

Determination of low-density lipoprotein (LDL) cholesterol

Low density lipoprotein (LDL) cholesterol concentration was calculated as the difference between total cholesterol and (HDL) cholesterol according to the method of **Skottovaet al. (1998)**.

LDL and vLDL were carried out by the following equations:

vLDL cholesterol (mg/ld) = (triglyceride / 5).

LDL cholesterol (mg/ld) = Total cholesterol - (vLDL + HDL cholesterol).

Lopez-virella et al., (1977).

Determination of serum glutamic oxalacetic transaminase (S.GOT) and serum glutamic pyruvic transaminase (S.GPT)

The activity of serum aspartate-aminotransferase (S.AST, commonly known as glutamic oxalacetic transaminase (S.GOT) and serum alanine aminotransferase (S.ALT), commonly known as glutamic pyruvic transaminase (S.GPT), were estimated according to **Varley et al. (1980)** using commercial kits produced by Pasteur Lab.

Determination of feed intake

Body weight, feed intake and faces extraction were measured every two days during six week test period, the amount of diet ingested was the difference between the weight of feed that rested in the feed bin (Da) and the amount placed one day before (D). These data were then used to calculate feed intake according to the following formula reported by **Ennouriet al. (2006)**.

$$\text{Feed intake (g)} = \frac{D - D_a}{1}$$

Where the number 1 correspond to the number of animals in the each cage.

Determination of digestibility

The digestibility of macronutrients was assessed as the relative difference between daily intake and 24 h faces excretion by following formula reported by **Ennouriet al. (2006)**.

$$\text{Digestibility} = \left\{ \frac{\text{Dietary intake} - \text{Fecal excretion}}{\text{Dietary intake}} \right\} \times 100$$

Determination of feed efficiency ratio

The feed efficiency ratio (FER) was determined by the following formula reported by **Ennouriet al. (2006)**.

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Weight gained (g)}}{\text{Feed offered (g)}}$$

Statistical analysis

The analysis of variance was carried out according to **Gomez and Gomez (1984)**. treatment means were compared by Duncan's multiple rang test (**Duncan, 1955**). All statistical analysis was performed using analysis of variance technique by means of "Costat" computer software package.

Results and Discussion

Biological experimental

Effect on blood glucose levels

Data given in Table (1) show the effect of feeding the prepared cookies on glucose levels of hyperglycemic rats, besides level of glucose in rats before induction are also reported correlation between glucose levels in each group after feeding for six weeks established to detect significant effect of feeding formulated diets on the glucose levels of hyperglycemic rats in comparison with glucose level of hyperglycemic rats that was fed on basal diet for the same six weeks.

Table (1): Effect of feeding albino rats on prepared diabetic cookies for six weeks on blood glucose levels

				1 st	2 nd	3 rd	4 th	5 th
Control:								
1	Control (-)	125.25 cd	120.00 b + 0.84	125.75 d + 1.02	87.50 e + 1.21	115.75 e + 0.34	110.75 e + 2.09	108.25 d ± 2.60
2	Control (+)	118.25 d + 1.83	187.75 d + 1.51	191.75 ab + 2.27	130.50 c + 0.15	164.50 bc	147.20 c + 1.12	153.00 b ± 1.08
Cookies:								
3	Control	119.25 d ± 2.14	215.50 bc	191.50 a ± 2.62	189.50 a	155.50 d ± 1.89	144.75 c ± 0.76	158.00 a ± 2.20
4	Cookies 1	103.75 e + 1.45	227.50 a + 2.45	194.50 ab + 2.31	157.50 b	165.00 b + 1.51	148.00 c + 1.54	159.50 a ± 1.66
5	Cookies 2	138.25 b + 2.87	226.00 a + 2.32	165.25 cd + 1.87	111.00 d	99.00 f + 1.44	119.00 d + 1.70	146.50 c ± 1.50
6	Cookies 3	130.75 c + 2.02	207.50 c + 1.21	193.75 ab + 1.66	192.00 a	158.00 c + 2.07	150.75 b + 2.04	148.75 c ± 2.30
7	Cookies 4	140.25 a + 1.65	204.25 c + 1.82	195.25 a + 2.05	194.25 a	179.50 a + 2.39	157.00 a + 1.32	149.75 a ± 1.06

- Each value is an average of four determinations

- **Group (1): Negative control fed on basal diet**
- **Group (2): Positive control fed on basal diet**
- **Groups (3: 7): diabetic rats groups fed on cookies**
- **Normal blood glucose level of rats ranged from 50 to 135 mg/dl**

Results in Table (1) indicate that glucose levels in plasma of the investigated rats before induction of hyperglycemia were ranged between 103.75 to 159.50 (mg/dl). This value was the normal range of glucose in adult rats plasma according to **Arun and Nalini (2002)**, who reported that the normal blood glucose level of rats ranged from 50 to 135 mg/dl. Also results in the same Table indicate that glucose levels of hyperglycemic rats, regardless of the diet used.

Extending feeding to six weeks on any investigated diets which contained plant sources such as seeds and leaves (black rice, lupine, germinated fenugreek, olive leaves and mulberry leaves) caused a continuous decrease in glucose levels of rats. Generally two weeks after rat's injection, it was noticed that blood glucose levels were clearly increased compared with control (+) which continue increasing to the end of the experimental.

Control (-) group showed the most lowest blood glucose levels at the end of six weeks (108.25 mg/dl) comparing with control (+) which was (153.00 mg/dl). Cookies 2 group which was (146.50 mg/dl) lowest blood glucose level after negative control (108.25 mg/dl). These mentioned diet groups contained (toasted fenugreek, mulberry leaves, stevia, lupine, germinated fenugreek, HFCS and olive leaves). These plant sources contained high amounts of fibers and lower blood glucose substances. **Mcintosh and Miller (2001)** reported that eating of diet containing fibers was accompanied with low level of plasma glucose.

Venancio et al., (2003) reported that protein isolate from some legumes has the same molecular mass amino acids sequence as that of bovin insulin. Furthermore, they showed that purified protein of legume reacts with antivertebrate insulin antibodies and lower blood glucose levels in diabetic animals. Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses. It's reported to have anti-diabetic, anti-fertility, anti-cancer, anti-microbial, anti-parasitic, hypo-cholesterolaemic effects (**Abbas, 2010**). Seeds of lupine (*Lupinustermis*) have hypoglycemic action in diabetic animals.

Mulberry powder with an optimized content of 1-deoxnojirimycin (DNJ) content, a compound shown to inhibit the action of the glucosidase enzyme that controls the digestion of carbohydrates. Mulberry leaves are more effective in controlling hyperglycemia (**Andallu and Vardacharyulu, 2001**). **Jemai et al., (2009)** reported that the main active component in olive leaf extract is oleuropein

presents significant hypoglycemic activity which essentially due to its antioxidant potential.

Several researches have been made to evaluate the hypoglycaemic effect of a lot of herbal drugs. **Alamet et al., (2009)** studied the combined effect of *Trigonellafoenumgraecum L.* there was a significant improvement in symptoms which may be due to good glycaemic control. The study also showed that reduction in blood sugar in test group as compared to control group. About 50% of diabetic individuals can be controlled by diet alone. But the better reduction in test group is due to low caloric diet and hypoglycaemic effect of *Trigonellafoenumgraecum* (Tukhum-e-Hulba).

Cookies 2 recorded (146.50 mg/dl) and that was the best lowest effect on reducing blood glucose levels, that may be due to containing 5% lupine seed flour, 5% germinated fenugreek flour, HFCS and olive oil which agree with **Pathak et al., (2000)** who mentioned that values of glucose level in food products being low because of contributory hypoglycemic effect of legumes and fenugreek seeds and agree also with **Sipsas (2008)** who reported that lupine fiber is colourless, odourless as well as a marked reduction in blood glucose levels.

It could be readily seen the effect of using lupine, germinated fenugreek seed flour, olive oil and HFCS on glucose levels in cookies 2 which was benefit for reducing glucose blood levels where it was (146.50 mg/dl) at the end of the six weeks compared with control (-) and (+) that may be due to containing high amounts of fibers and low blood glucose substances. Dietary supplementation with the anthocyanin rich extract from black rice prevented the development of fructose-induced insulin resistance (**Guo et al., 2006**). These results are in agreement with those obtained by **Elhadidy, (2009)**.

In this respect **Ba-Jaber et al., (1997)** reported that high carbohydrates high fiber low fat diets have a strong effect on fasting plasma glucose reduction. **Shiyou and Liang (2001)** studied that the dietary fibers lowered post prandial serum glucose level at least by three mechanisms. First, they increase the viscosity of small intestine juice and hinder diffusion of glucose. Second, they bind glucose and decrease the concentration of available glucose in small intestine and the third one; they retard alpha amylase action. All of these previous mechanisms decrease the absorption of glucose and the concentration of postprandial serum glucose.

Savita et al., (2004) reported that the plant based stevia herb is a low calorie nutritious component has an immense potential in the main stream of food processing industries as a health and diabetic benefactor. Glycemic index of selected products found to be lower in diabetics as well as in normal individuals.

Olive leaves are considered as a cheap raw material which can be used as a useful source of high-added value products (**Jemaiet *et al.*, 2009**).

Body weight gain, food intake and digestibility of albino rats fed on prepared diabetic cookies

From Table (2), it is clear that alloxan injection did not cause any significant decrease in the feed intake after six weeks when compared with the normal rats (17.75 g/day). Unchanging in feed intake of alloxanized diabetic rats did not affecting directly on a gain weight and consequently on feed efficiency ratio. For the fact, the food consumption was unchanged in case of hyperglycemic or hypoglycemic rats as well as normal control but the gain of body weight was decrease relative to control (+). Since insignificant changes in food consumption was not parallel to the growth of rats agree with **Ennouriet *et al.*, (2006)**.

Table (2): Body weight gain and food intake of albino rats fed on prepared diabetic cookies.

No	Rat groups	Body weight gain (BWG) %	Food intake (FI) gm	Feed efficiency ratio (FER) %	Fecal mean %	Digestibility
Control:						
1	Control (-)	35.75 e ±	17.75 a ±	2.02 c ±	2.11 a ±	88.12 a ±
2	Control	-17.67 f ±	17.68 a ±	-0.99 d ±	1.95 a ±	88.92 a ±
Cookies:						
3	Control	51.72 ab ±	16.94 c ±	3.05 a ±	2.11 a ±	87.54 a ±
4	Cookies 1	35.44 e ±	17.48 b ±	2.03 c ±	2.00 a ±	88.56 a ±
5	Cookies 2	55.89 a ±	17.62 a ±	3.18 a ±	2.04 a ±	88.40 a ±
6	Cookies 3	41.44 d ±	17.30 b ±	2.39 b ±	2.02 a ±	88.31 a ±
7	Cookies 4	45.17 c ±	17.60 a ±	2.56 b ±	2.07 a ±	88.25 a ±

It was noticed that rats group fed on control (-) was the highest body weight gain (35.75) with being significantly different with rats groups fed on cookies comparing with control (+) showed the lowest BWG (-17.67), generally due to insulin deficiency and increased lypolysis (**Youssef *et al.*, 2007**). Data in Table (2) cleared that diets containing stevia sweetener increased BWG agree with **Abdel-Rahim *et al.*, (2004)** who showed the effect of feeding on biscuits sweetened with stevioside on body weight gain and feed intake of the experimental rats by

increasing BWG. It may be concluded that all treatments affected positively the BWG% comparing with control positive group.

For food daily intake, it was clearly that it increased in all treated groups comparing with control positive group (17.68) with being cookies 2 the highest food intake (17.62) comparing with control negative (17.75) agree with **Abdel-Rahim et al., (2004)**. It is worth mentioning that the treated diabetic groups with plant sources such as seeds and leaves rich with fibers resulted in pronoun observed increasing of feed efficiency ratio comparing control positive group (-0.99), where FER ranged between (2.02 to 3.18) in cookies rat groups comparing with control (-). For the fact, the food consumption was unchanged in case of hyperglycemic or hypoglycemic rats as well as normal control but the gain of body weight was decrease relative to control (+). Since insignificant changes in food consumption was not parallel to the growth of rats agree with **Ennouriet al., (2006)**.

There was no significantly differences between all rat groups for fecal mean comparing with positive control which recorded (1.95). Up on the data in Table (2) apparent that digestibility was high in all groups with being no significantly different between all groups and the controls. These results are in the same range with those mentioned by **Pathak et al., (2000)**, **Abdel-Rahim et al., (2004)** and **Kochharet al., (2008)**.

Effect of feeding albino rats on prepared cookies for six weeks on serum lipids

Blood samples were collected from rats, after 6 weeks of dietary treatments, for analyses of triglycerides (TG), total cholesterol (TC) and lipoprotein cholesterol (high-density lipoprotein cholesterol, HDL-C concentrations) and the results are given in Table (3).

No	Rat groups	Total Cholesterol* (mg/dl)	HDL-c* (mg/dl)	T.G* (mg/dl)	VLDL-c** (mg/dl)	LDL-c*** (mg/dl)	LDL/HDL (mg/dl)
Control :							
1	Control (-)	121.21 d +	53.80 a +	163.11 a +	32.62 a +	34.97 e +	0.65 c + 1.34
2	Control(+)	133.14 c ±	43.08 b ±	113.25 e ±	22.65 c ±	66.69 c ±	1.54 b ± 1.91
Cookies:							
3	Control	139.43 c ±	41.92 c ±	154.72 b ±	30.94 a ±	92.25 a ±	2.20 a ± 1.00
4	Cookies 1	165.11 a ±	39.64 d ±	138.09 c ±	27.62 b ±	97.85 a ±	2.47 a ± 1.14
5	Cookies	142.73 b ±	41.50 c ±	124.60 d ±	24.92 b ±	76.31 b ±	1.84 b ± 1.51
6	Cookies	123.82 d ±	45.87 b ±	128.78 d ±	25.76 b ±	52.19 d ±	1.14 b ± 1.61

7	Cookies	147.42 b ±	43.05 b ±	152.61 b ±	30.52 a ±	73.85 b ±	1.72 b ± 1.2
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Table (3): Effect of feeding albino rats on prepared diabetic cookies for six weeks on serum lipid*.

* Each value is an average of four determinations.

** VLDL cholesterol (mg/dl) = (Triglyceride / 5).

*** LDL cholesterol (mg/dl) = Total cholesterol – (VLDL+HDL cholesterol).

Although the relationship between lipids pattern abnormalities and diabetes is complex, there is usually a specific lipids pattern abnormality found in diabetes (**Rosalyn and Bauman, 1983**). It was reported that hypertriglyceridemia, hypercholesterolemia and reduced HDL-c levels are commonly observed in diabetes. Normal values in human should be in the range of: Total triglycerides (50 to 250 mg/dl), LDL-c (< 160 mg/dl), TC (below 200 mg/dl) and HDL-C (above 45 mg/dl) (**Baur, 1995**). Using olive oil reduced Total cholesterol, LDL, HDL (**Liu and Tsai, 2002**). The administrations of olive oil showed a better profile in the lipid as well as decrease in the concentration of lipid hydroperoxides either in normal or diabetic rats (**Alhazza, 2007**). Glucose cholesterol levels, triglyceride, HDL and LDL concentration decrease significantly ($p < 0.01$) in rats treated with olive oil after six weeks.

Data in Table (3) refer to values of lipids profile for positive control group and negative control group as well as for different diabetic rat groups fed on diabetic diets from plant sources such as seeds and leaves.

Effect of prepared cookies on serum total cholesterol levels

It was noticed in Table (3) that the highest cholesterol level was found to be in rats group fed on cookies 1 as (165.11 mg/dl), that may be due to containing butter more than the two controls (+) and (-) as (133.14 mg/dl) and (121.21 mg/dl), respectively. Cookies 3 had low content of cholesterol (123.82 mg/dl) that may be due to containing 10% germinated lupine seed flour, 10% fenugreek powder and olive oil. Generally, all treatments effected positively on serum total cholesterol where it showed a great decrease comparing with negative and positive control, agree with **Abd-Elhady (2008)** who showed that rats fed on diets containing antioxidant extracted from defatted black rice bran had lower serum total cholesterol (TC). These results are in a harmony with those mentioned by **Alhazza, (2007)** and **Elhadidy, (2009)**.

In this respect **Kritchevsky and story, (1993)** reported many mechanisms by which fiber may affect cholesterol levels which involve aspects of bile acids metabolism, including inhibition of absorption cholesterol due to binding of bile acids.

Effect of prepared cookies on HDL-c levels

Induction of rats by alloxan caused a highly significant increase in HDL-c (39.64 mg/dl to 53.80 mg/dl). Checking the serum HDL levels, there was significantly different between rat groups fed on all diets and the two controls. Feeding on diabetic cookies caused significantly increasing in HDL-c. The highest HDL-c was found to be in control cookies followed by cookies 3 as (53.80 mg/dl) and (45.87 mg/dl), respectively. These results agree with **Ennouriet al., (2006)**.

Most of highest HDL-c values were found in rats groups fed on diets contained olive oil such as cookies 2. That was disagreeing with saying that using olive oil reduced total cholesterol, LDL, HDL (**Liu and Tsai, 2002**). The administration of olive oil showed a better profile in the lipid as well as decrease in the concentration of lipid hydroperoxides either in normal or diabetic rats (**Alhazza, 2007**). Glucose cholesterol level, triglyceride and LDL concentration decrease significantly ($p < 0.01$) in the rats treated with olive oil after six weeks. Olive oil highly enriched in oleic acid (unsaturated fatty acid) caused increase in HDL. It is known that oleic and linoleic acids have beneficial health effects including alleviating cardiovascular complaints, inflammatory condition, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases. Linoleic acid is an essential fatty acid and a precursor of arachidonic acid biosynthesis, the substrate for eicosanoid synthesis. It has long been accepted as having hypocholesterolemic effects.

Effect of prepared cookies on LDL-c levels

We can observe from Table (3), the effect of different treatments on serum LDL-c after feeding of hyperglycemic rats for six weeks. All treatments decreased serum LDL levels at different degrees (34.97 to 97.85 mg/dl). The highest LDL-c was found to be in rat groups fed on cookies 1 as (97.85 mg/dl) compared with control (-) and (+) as (34.97 mg/dl) and (66.69 mg/dl), respectively. It was noticed that rats group fed on control (-) was the lowest LDL-c content (34.97 mg/dl).

The administration of olive oil showed a better profile in the lipid as well as decrease in the concentration of lipid hydroperoxides either in normal or diabetic rats (**Alhazza, 2007**). Olive oil highly enriched in oleic acid (unsaturated fatty acid) caused that decrease. Glucose, cholesterol levels, triglycerides, HDL and LDL concentrations decrease significantly ($p < 0.01$) in the rats treated with olive oil after six weeks.

Effect of prepared cookies on serum triglycerides

The effect of different treatments on serum triglycerides after feeding of hyperglycemic rats for six weeks showed that all treatments decreased serum triglycerides levels at different degrees (113.25 to 163.11 mg/dl).

There was significantly different between all rats groups that may be due to using different ingredients, oil and fat. It was clear that the highest TG content was found to be (163.11 mg/dl) in control (-) group followed by rats group fed on control cookies.

Deserve to notice that rats fed on control (-) were the highest TG content (163.11 mg/dl) that may be due to containing high amount of oil and fat due to composition and frying process. The lowest TG content was found in control (+) which was (113.25mg/dl) followed by (124.60 mg/dl) for rats group fed on cookies 2. The administration of olive oil showed a better profile in the lipid as well as decreases in the concentration of lipid hydroperoxides either in normal or diabetic rats (Alhazza, 2007). Glucose cholesterol level, triglyceride, HDL and FDL concentration decrease significantly ($p < 0.01$) in the rats treated with olive oil after six weeks.

Effect of plasma transaminases activities

The assay of enzyme levels in the extra cellular body fluid, such as blood serum, is important aid to clinical diagnosis and management of disease. Measurements of the changes in enzyme levels offer a degree of injury, than is possible using the other clinic chemical parameters. Most significant for the development of diagnostic enzymology were the studies on the transaminases, particularly glutamic-pyruvic transaminase (GPT) or alanin aminotransferase (ALT) and glutamic oxaloacetic transaminase (GOT) or aspartate aminotransferase (AST). In all liver dysfunction, the (GPT) and (GOT) levels are increased in serum, the extents giving a useful differential index of the type of dysfunction. The activity of these enzymies was found to be elevated in serum after myocardial and liver disease (Foster, 1980).

Table (4): Effect of fed albino rats on diabetic diets for six weeks on plasma transaminases activities*

No	Rat groups	GOT (IU/L)**	GPT (IU/L)***	GOT/GPT
Control:				
1	Control (-)	40.13 e + 1.77	20.71 d +	1.94 b +
2	Control (+)	60.75 c ± 1.93	28.53 c ±	2.13 a ±

Cookies:				
3	Control	58.19 d + 1.37	27.52 c +	2.12 a +
4	Cookies 1	62.37 b ± 1.15	38.51 a ±	1.62 b ±
5	Cookies 2	61.08 b + 1.60	32.18 bc+	1.90 b +
6	Cookies 3	61.77 b ± 1.22	29.85 c ±	2.07 a ±
7	Cookies 4	69.50 a + 1.95	33.07 b +	2.10 a +

*Each value is an average of four determinations.

** GOT (glutamic oxalacetic transaminase).

*** GPT (glutamic pyruvic transaminase).

Interpretations of the changing enzyme levels could only be made if the normal ranged of enzyme activities in serum are be known. In healthy humans, the concentration of cellular enzymes in the extra cellular fluids is fairly low, ranging between 5-30 mu/ml (Foster, 1980 and Louz, 1997).

It was noticed from Table (4) that the highest GOT content was (69.50) found in rat groups fed on cookies 4. Deserve to notice that rats fed on cookies were the highest GOT content (from 40.13 to 69.50). The lowest GOT content (40.13) was found to be in control (-) that may be due to containing high amount of plans (10% germinated fenugreek, 10% olive leaves and stevia sweetener) without using any oil or fat. It was clear that diets containing stevia leaves powder, aqueous extract and steviosoid led to gradual reduction in plasma GPT (ALT) and GOT (AST) levels (Youssef *et al.*, 2007 and Junbi and Amer, 2010).

For GPT it could be also noticed that all rat groups fed on control cookies recorded values less than that of control positive (28.53) as (27.52), respectively. Rats groups fed on diabetic children cookies containing lupine seed flour were low in GOT content that my be due to lupine improved liver function by decreasing the activities of AST and ALT. With compared to untreated diabetic animals indicating that mulberry leaves controlled the rate of gluconeogenesis (Andallu and Vardacharyulu, 2001). These results were at the same line with those obtained by Alam (2001), Abdel-Rahim *et al.*, (2004) and Elhadidy (2009).

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التقييم البيولوجي لكوكيز الأطفال مرضى السكر المعد من مصادر نباتية

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المستخلص العربي

يهدف هذا البحث لاعداد كوكيز ذات قيمة غذائية عالية وذلك للأطفال مرضى السكر ولخفض نسبة سكر الجلوكوز بالدم. تم إعداد أربع أنواع من الاسناكس للأطفال مرضى السكر عالية القيمة الغذائية باستخدام بذور النباتات (الأرز الأسود والترمس والحلبة المنبتة) والأوراق (التوت، الزيتون). حيث أجريت دراسة تأثير استبدال السكر على الخصائص البيولوجية للكوكيز المعد باستخدام مستويات مختلفة من المحليات الطبيعية والصناعية (ستيفيا وشراب الذرة عالي الفركتوز) واستبدال الزبدة بزيت الزيتون.

تم توزيع الفئران عشوائيا على (7 مجموعات) أربعة فئران لكل منهما، وأدى استمرار تغذية فئران التجارب لسته أسابيع على أي من الكوكيز المحتوي على مصادر نباتية مثل البذور والأوراق لانخفاض مستمر في مستوى الجلوكوز لدى الفئران. مثلت كوكيز 2 (146.50 mg/dl) أدنى مستوى جلوكوز في الدم بعد 6 اسابيع مقارنة بمجموعة الفئران الضابطة السالبة والموجبة. وقد لوحظ أن مجموعة الفئران الضابطة السالبة هي الأعلى قيمة للوزن المكتسب (35.75) مع وجود فروق معنوية بين كل مجموعات الفئران المتغذية على الكوكيز مقارنة بمجموعة الفئران الضابطة الموجبة التي انخفض فيها الوزن المكتسب (-17.67). بالنسبة للغذاء المتناول، فقد كان واضحاً زيادته في كل المعاملات مقارنة بمجموعة الفئران الضابطة الموجبة (17.68) مع كون المجموعة الضابطة السالبة هي الأعلى لتناول الغذاء (17.75). تواجبت أعلى قيمة للكفاءة الغذائية (3.18) في مجموعة الفئران المتغذية على كوكيز 2 مقارنة مع المجموعة الضابطة التي سجلت قيمة كفاءة غذائية (2.02).

سجلت المجموعة الضابطة السالبة اقل نسبة للكوليستيرول (121.21 mg/dl) يليها مجموعة الفئران المتغذية على كوكيز 3 (123.82 mg/dl). أدت جميع المعاملات الى انخفاض مستويات الكوليستيرول منخفض الكثافة، كما تواجد أعلى محتوى للكوليستيرول مرتفع الكثافة (53.80 mg/dl) في المجموعة الضابطة السالبة تليها (53.80 mg/dl) لمجموعة الفئران المتغذية على كوكيز 3 مقارنة مع المجموعة الضابطة الموجبة التي سجلت (66.96 mg/dl)

تواجد أعلى محتوى للـ (69.50) GOT لدى مجموعة الفئران المتغذية على كوكيز ، كما أشارت هذه النتائج إلى أن تغذية الفئران على كوكيز الاطفال مرضى السكر المعد باستخدام المصادر النباتية سألفة الذكر



اثر ايجابي على مستوى الكوليستيرول عالي الكثافة وسلبيا على مستوى الكوليستيرول منخفض الكثافة والدهون الثلاثية كما ادى الى تحسن في وظائف الكبد.
الكلمات المفتاحية: الفحص البيولوجي، الأطفال المصابون بالسكر، الكوكيز، مصادر نباتية، انزيمات الكبد.