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Biological Assay of Diabetic Children Snacks Prepared from Plant Sources

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Abstract

This work was aimed to prepare high nutririve value snacks for diabetic children and as ablood sugar lowering materials. Four snacks 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.

Extending feeding albino rats for six weeks on any investigated snacks which contained plant sources such as seeds and leaves caused a continuous decrease in glucose level of rats. Snack 2 groups which were (120.25 mg/dl) showed lowest blood glucose level after nigative control (108.25 mg/dl). 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 snacks 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 control positive group (17.68) with being snack 2 had the highest food intake (17.97) comparing with control negative (17.75). FER ranged between 1.64 to 1.96 in snack rat groups comparing with control (-). Cotrol snack were the highest FER comparing with control negative which recorded (2.02).

Snack 1 had low content of cholesterol (97.05 mg/dl). The highest HDL-C was found to be in control snacks fellowed by snacks 2 as (55.36 mg/dl) and (54.32 mg/dl), respectively. All treatments decreased serum LDL levels at different degrees (22.18 to 95.48 mg/dl). Rats groups fed on snack 2 were the lowest LDL-C content (22.18 mg/dl). The highest GOT content was (76.62) found in rat groups fed on snack 3. All rat groups fed on snack 1 recorded value less than that of control positive (28.53) as (27.19), respectively. 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, Snacks, Plant Sources, (GOT) and (GPT).

Introduction

Diabetes mellitus (DM) is a chronic metabolic disease with the highest rates of prevalence and mortality world wide that is caused by an absolute or relative lack of insulin and or reduce insulin activity **(Jemai *et 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 **(Sanehez *et 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 **(Safdar *et 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 arteriosclerosis, and is a type of functional food **(Koguchi *et 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 **(Magni *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, fats, 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 **(Jemai *et al.*, 2009).**

Olive oil is the principal source of added lipid in the Meditranean 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-cilic-diterpene steriol (Steviolbioside, stevioside rebaudiosides 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 sweetener is commonly used as nutritive and posses a high-intensty 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).**

Consumption of wide variety of fast foods, including convenience and snack foods continues to increase in the USA and other countries **(Stern and Denenberg, 1980 and Morris, 1982).** The preference shown by children and teenagers towards these products is evident **(Ekvall and Vallo, 1983).** The frequency of snacks consumption of children aged 7-18 years old in Bangkok (Thailand) was found to be 51.3% consuming every day **(Sinthavalai, 1984).**

Extruded snack products are predominantly made from rice flour or starch and tend to be low in protein and have a low biological value, as they have a low concentration of essential amino acids. A great deal of attention has been paid to fortifying the extruded food with cereals high in protein and lysine to improve the essential amino acids contents. Generally, cereals and legumes, such as red kidney beans, soy and corn have been used to make highly nutritional products **(Baskaran and Bhattacharaya, 2004).** Snacks have become an integral part of the daily food intake of the majority of the world's population. Basically, they are prepared from natural ingredients or components according to predesigned plans to produce with specified quality **(Limsangouan *et al.*, 2010).**

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

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 ºC 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 forgein 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 ºC for 96 hours, milled and kept in polyethylene bags until used**.** Olive and mulberry leaves were cleaned thoroughly by washing. Then, cleaned leaves were minced; sun dried and 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 ºC 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 spereaded 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 ºC) up to three days **(El-Bagoury, 2005).** Oven air (60-70 ºC) was used for 48 hours to dry the lupine, germinated and toasted fenugreek seeds **(Sidkey *et al.*, 1993),** then all plants were grounded in an electric mill and sieved at in 120 mesh to produce a fine powder. All samples were kept in polyethylene bags at 5 ºC until blended.

Preparation of diabetic children snacks using plant seeds and leaves

Four formulas of snacks 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 snacks 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 snack

Ingredients	Snacks

Flour (mixtures + wheat)	(gm)	100
Sugar	(gm)	12.5
Egg	(ml)	23.9
Butter	(gm)	25
Yeast	(gm)	2.5
Vanilla	(gm)	0.03

Table (A) show the content of control snacks made from 100% wheat flour. The dry ingredients were mixed thoroughly for one minute by hand. The yeast and sugar dissolved in warm water (50 ml, 50 ºC), butter was added to the flour and cut in until the butter was broken into pieces. The yeast sugar mixture and egg were then added, mixed manually for 2 min and fermented for 2 h. The dough was shaped by new modification using (koma), fried the shaped snacks directly in corn oil **(Akubor 2004).**

Table (B): The composition of diabetic children snacks

Diabetic Snacks	Wheat flour %	Percentage of plant additive	Sweeteners	Fats
Control	100	------	Sucrose	Butter
Snack 1	80	20 (10% Lupine + 10% Olive leaves)	Sucrose	Olive oil
Snack 2	90	10 (5% Lupine + 5% Black rice)	Stevia powder	Olive oil
Snack 3	90	10 (5% Lupine + 5% Mulberry leaves)	HFCS	Olive oil
Snack 4	70	30 (15% Lupine + 15% Germinated fenugreek)	Sucrose	Butter

Snacks

The dry ingredients were mixed thoroughly for one minute by hand. The yeast and sugar dissolved in warm water (50 ml, 50 ºC), butter was added to the flour and cut in until the butter was broken into pieces. The yeast sugar mixture and egg were then added, mixed manually for 2 min and fermented for 2 h. The dough was shaped by new modification using (koma), fried the shaped snacks directly in corn oil **(Akubor 2004).**

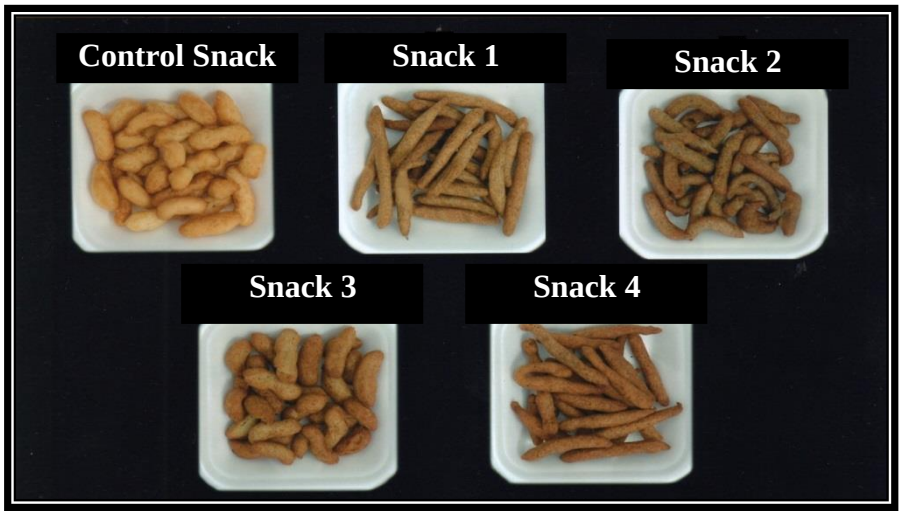

Fig (1): The best sensory evaluated diabetic children snack.

Control snack: 100 % wheat flour.
Snack (1): 80 % wheat flour, 10 % lupine seed flour, 10 % olive leaves, sucrose and olive oil.
Snack (2): 90 % wheat flour, 5 % lupine seed flour, 5% black rice, stevia leaves powder and olive oil.
Snack (3): 90 % wheat flour, 5 % lupine seed flour, 5% mulberry leaves, HFCS and olive oil.
Snack (4): 70 % wheat flour, 15 % lupine seed flour 15% germinated fenugreek flour, sucrose and butter.

Biological evaluation of prepared snacks

Animals

White male albino rats Sprague Dawely (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 (5 groups) four rats each. Each animal was individually housed in a wire bottomed, stainless steel cage under the normal condition. The animals were weighted every week 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 hour 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 snacks 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 snacks.
G4: Rats fed on snacks 20 % (lupine and olive leaves) used sucrose and olive oil.
G5: Rats fed on snacks 10 % (black rice and lupine) used stevia and olive oil.
G6: Rats fed on snacks 10 % (lupine and mulberry leaves) used HFCS and olive oil.
G7: Rats fed on snacks 30 % (lupine and germinated fenugreek) used sucrose 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:

BWG % = (Final Weight – Initial / Initial Weight) × 100.
FER = [Gain in body (g/day)/ Food intake (g/ day) × 100].
Food Intake = Rat body weight × (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 remaind is corn starch (69.5 %), according to **Campbll (1963).**

Determination of rats blood glucose

Blood glucose was measured according to the method described by **Alles *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 colt. 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 º 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 with out 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 **Skottova *et 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 **Ennouri *et al.* (2006).**

$$\text{Feed intake (g)} = \left(\frac{D - Da}{1}\right) / 1$$

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 **Ennouri *et al.* (2006).**

Digestibility = {(Dietary intake – Fecal excretion)/ Dietary intake} × 100

Determination of feed efficiency ratio

The feed efficiency ratio (FER) was determined by the following formula reported by **Ennouri *et 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 snacks 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 snacks for six weeks on blood glucose levels

No.	Rat groups	Before treatment (after 24 hr .fasting)	After injected with alloxan	Weekly blood glucose level mg/dl				
				1 st	2 nd	3 rd	4 th	5 th
Control:								

1	Control (-)	125.25 a-c ± 1.70	120.00 c ± 0.84	125.75 d ± 1.02	87.50 f ± 2.89	115.75 cd ± 1.72	110.75 d ± 1.62	108.25 e ± 2.19
2	Control (+)	118.25 a-d ± 1.83	187.75 ab ± 1.51	191.75 ab ± 2.27	130.50 de ± 1.80	164.50 ab ± 3.56	147.20 a-d ± 2.53	153.00 ab ± 3.01
Snacks:								
3	Control	138.50 ab ± 2.15	221.75 a ± 1.86	194.75 a ± 2.93	173.50 a-c ± 3.71	182.25 a ± 2.04	153.00 ab ± 1.99	160.00 a ± 1.08
4	Snack 1	146.50 a ±1.90	213.00 a ± 2.19	191.25 ab ± 3.04	179.00 a-c ± 2.10	138.00 a-d ± 2.91	164.25 a ± 2.55	159.75 a ± 1.41
5	Snack 2	152.25 a ± 2.65	214.25 a ± 3.06	146.50 cd ± 1.61	125.00 de ± 1.15	98.25 d ± 1.40	113.00 cd ± 2.17	120.25 c-e ± 3.17
6	Snack 3	126.50 a-c ± 2.01	222.50 a ± 1.46	190.75 ab ± 1.07	171.50 a-c ± 2.08	148.00 a-c ± 3.03	144.50 a-d ± 1,.13	147.00 a-d ± 3.93
7	Snack 4	126.75 a-c ± 1.82	204.75 ab ± 1.50	97.75 a ± 2.86	187.25 a-c ± 1.39	169.50 ab ± 0.62	158.75 a ± 1.11	162.00 a ± 2.85

- 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 Snacks
- 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 (118.25 mg/dl) to (152.25 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 level 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 comparing with control (+) which was (153.00 mg/ld). Snack 2 groups which were (120.25 mg/dl) lowest blood glucose level after nigative 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. Further more, they showed that purified protein of legume reacts with antiverteabate 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 *(Lupinus termis)* 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 carbhydrates. 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 hypoglycemiant activity which essentially due to its antioxidant potential.

Several researches have been made to evaluate the hypoglycaemic effect of a lot of herbal drugs. **Alam *et al.*, (2009)** studied the combined effect of *Trigonella foenum graecum 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 caloriec diet and hypoglycaemic effect of Trigonella foenum graecum (Tukhum-e-Hulba).

It could be readily seen the effect of using lupine seed flour, black rice flour, olive oil and stevia on glucose levels in snack 2 which was benefit for reducing glucose blood levels where it was (120.25 mg/ld) at the end of the six weeks compared with control (-) and (+) that may be due to containing high amounts of fibers and lower blood glucose substances. Dietry 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 diet have a strong effect on fasting plasma glucose reduction. **Shiyiou 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 alfa 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 **(Jemai *et al.*, 2009).**

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

From the 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 ration. 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 **Ennouri *et al.*, (2006).**

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

No	Rat groups	Body weight gain (BWG) %	Food intake (FI) gm	Feed efficiency ratio (FER) %	Fecal mean %	Digestibility
Control:						
1	Control (-)	35.75 a ± 2.09	17.75 c ± 1.13	2.02 a ± 6.17	2.11 a ± 3.08	88.12 a ± 1.40

2	Control (+)	-17.67 f ± 5.98	17.68 c ± 4.25	-0.99 f ± 3.05	1.95 a ± 2.20	88.92 a ± 3.37
Snacks:						
3	Control	34.41 b ± 4.63	17.74 c ± 1.87	1.94 b ± 2.51	1.92 a ± 6.01	89.18 a ± 1.95
4	Snack 1	35.04 a ± 4.09	17.82 ab ± 3.42	1.96 b ± 1.18	1.99 a ± 1.56	88.81 a ± 1.15
5	Snack 2	32.81 c ± 6.29	17.97 a ± 7.10	1.83 c ± 1.71	1.82 a ± 5.08	89.85 a ± 3.70
6	Snack 3	29.04 e ± 5.62	17.76 b ± 4.81	1.64 e ± 5.14	1.96 a ± 6.03	88.94 a ± 7.52
7	Snack 4	30.54 d ± 1.41	17.77 b ± 1.70	1.72 d ± 3.09	1.85 a ± 1.58	89.59 a ± 2.30

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 snacks 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 snack 2 the highest food intake (17.97) comparing with control negative (17.75) agree with **Abdel-Rahim *et al.*, (2004).** It is worthy 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 (-.099), where FER ranged between (1.64 to 1.96) in snack rat groups comparing with control (-). Cotrol snack were the highest FER comparing with control negative which recorded (2.02) agree with **Ennouri *et al.*, (2006).**

There was no significantly difference 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 Kochhar *et al.*, (2008).**

Effect of feeding albino rats on prepared snacks for six weeks on serum lipid

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).

Table (3): Effect of feeding albino rats on prepared diabetic diets for six weeks on serum lipid*.

No	Rat groups	Cholesterol* (mg/dl)	HDL* (mg/dl)	T.G*	VLDL** (mg/dl)	LDL*** (mg/dl)	LDL/HDL (mg/dl)
Control :							
1	Control (-)	121.21 e ± 2.05	53.80 c ± 5.17	163.11 c ± 6.01	32.62 c ± 1.72	34.97 e ± 5.04	0.65 f ± 4.65
2	Control(+)	133.14 d ± 4.87	43.08 d ± 5.60	113.25 f ± 2.25	22.65 f ± 7.09	66.69 b ± 4.36	1.54 b ± 1.11
Snacks :							

3	Control	150.13 b ± 2.21	55.36 a ± 3.12	159.31 d ± 6.77	31.86 d ± 3.37	62.91 c ± 7.13	1.14 c ± 3.01
4	Snack 1	97.05 g ± 7.18	43.12 d ± 2.01	158.75 d ± 4.30	31.75 d ± 1.10	22.18 f ± 5.79	0.51 g ± 2.50
5	Snack 2	141.81 c ± 3.95	54.32 b ± 6.18	146.48 e ± 1.03	29.30 e ± 2.15	58.19 d ± 3.12	1.07 d ± 4.43
6	Snack 3	112.19 f ± 6.43	46.14 d ± 1.15	165.17 b ± 9.52	33.03 b ± 6.12	33.02 e ± 3.76	0.72 e ± 1.14
7	Snack 4	170.24 a ± 3.14	40.50 e ± 4.19	171.28 a ± 2.04	34.26 a ± 6.92	95.48 a ± 3.05	2.36 a ± 2.34

*** Each value is an average of four determinations.**
**** VLDl cholesterol (mg/ld) = (Triglyceride / 5).**
***** LDL cholesterol (mg/ld) = 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 lipid 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 snacks 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 snack 4 as (170.24 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. Snack 1 had low content of cholesterol (97.05 mg/dl) that may be due to containing 10% lupine seed flour, 10% olive leaves 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 snacks on HDL-cholesterol levels

Induction of rats by alloxan caused a highly significant increase in HDL-C (43.08 mg/dl to 55.36 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 snacks caused significantly increasing in

HDL-C. The highest HDL-C was found to be in control snacks fellowed by snacks 2 as (55.36 mg/dl) and (54.32 mg/dl), respectively. These results agree with **Ennouri *et al.*, (2006).**

Most of highest HDL-C values were found in rats groups fed on diets contained olive oil such as snack 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, triglycerideand 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) cased increase in HDL.

It is know 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 snacks on LDL-cholesterol levels

We can observe from Table (3), the effect of different treatments on serum LDL cholesterol after feeding of hyperglycemic rats for six weeks. All treatments decreased serum LDL levels at different degrees (22.18 to 95.48 mg/dl). The highest LDL-C was found to be in rat groups fed on snack 4 as (95.48 mg/dl compared with control (-) and (+) as (34.97 mg/dl) and (66.69 mg/dl), respectively. It was noticed that rats groups fed on snack 2 were the lowest LDL-C content (22.18 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 snacks 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 171.28 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 (171.28 mg/dl) in rats group fed on snack 4 containing 15% lupine seed flour, 15% germinated fenugreek flour and butter) followed by rats group fed on snacks 3 which was (165.17 mg/dl)) containing 5% lupine seed flour, 5% mulberry leaves and olive oil comparing with control (-) which was (163.11 mg/dl).

Deserve to notice that rats fed on snacks were the highest TG content (from 146.48 to 171.28) that may be due to snack which contained 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). 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 ton 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 f ± 3.24	20.71 f ± 5.07	1.94 d ± 1.20
2	Control (+)	60.75 e ± 1.67	28.53 d ± 7.10	2.13 b ± 3.74
Snacks :				
3	Control	64.65 c ± 1.15	35.76 b ± 4.95	1.81 f ± 1.96
4	Snack 1	59.87 d ± 2.09	27.19 e ± 4.16	2.20 a ± 5.14
5	Snack 2	67.00 b ± 6.34	35.50 b ± 0.82	1.89 e ± 8.03
6	Snack 3	76.62 a ± 3.51	40.39 a ± 1.66	1.90 e ± 6.70
7	Snack 4	66.21 bc ± 1.06	32.10 c ± 3.91	2.06 c ± 1.48

*** 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 (76.62) found in rat groups fed on snack 3. Deserve to notice that rats fed on snack were the highest GOT content (from 59.87 to 76.62) that may be due to snacks which contained high amount of oil and fat due to composition and frying process. 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) with out 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 snack 1 recorded values less than that of control positive (28.53) as (27.19), respectively. Rats groups fed on diabetic children snacks 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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مجلة التربية النوعية والتكنولوجيا (بحوث علمية وتطبيقية)
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التقييم البيولوجي لاسناكس الأطفال مرضى السكر المعد من مصادر نباتية

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المستخلص العربي

يهدف هذا البحث لاعداد اسناكس ذات قيمة غذائية عالية وذلك للأطفال مرضى السكر ولخفض نسبة سكر الجلوكوز بالدم. تم إعداد أربع انواع 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 للاسناكس المعد باستخدام مستويات مختلفة من المحليات الطبيعية والصناعية (ستيفيا وشراب الذرة عالي الفركتوز) واستبدال الزبدة بزيت الزيتون.

أدى استمرار تغذية فئران التجارب لستة أسابيع على أي من الاسنكاس المحتوي على مصادر نباتية مثل البذور والأوراق لانخفاض مستمر في مستوى الجلوكوز لدى الفئران. مثلت اسناكس2 (120.25 mg/dl) أدنى مستوى جلوكوز في الدم بعد مجموعة الفئران الضابطة السالبة (108.25 mg/dl). وقد لوحظ أن مجموعة الفئران الضابطة السالبة هي الأعلى قيمة للوزن المكتسب (35.75) مع وجود فروق معنوية بين كل مجموعات الفئران المتغذية على الاسناكس 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 اسناكس2 هو الاعلى تناولا للغذاء (17.97) مقارنة بمجموعة الفئران الضابطة السالبة (17.75).

تراوحت قيم الكفاءة الغذائية بين (1.64 الى 1.96) في مجموعات الفئران المتغذية على الاسناكس مقارنة بمجموعة الفئران الضابطة السالبة. مثل اسناكس1 اعلى قيمة للكفاءة الغذائية (1.96) بعد مجموعة الفئران الضابة السالبة (2.02). قدم اسناكس1 اقل قيمة للكولسترول بواقع (97.05 mg/dl). تواجدت اعلى قيمة للكوليستيرول مرتفع الكثافة في اسناكس الكنترول يليه اسناكس2 بواقع (55.36 و 54.32 ملجم/لتر دم) على التوالي.

أدت جميع المعاملات الى انخفاض مستويات الكوليستيرول منخفض الكثافة بين (22.18 و 95.48 ملجم/لتر دم). وكانت مجموعة الفئران المتغذية على اسناكس2 هي ادنى قيمة للكولستيرول منخفض الكثافة (22.18 mg/dl). أدت تغذية فئران التجارب على اسناكس الاطفال مرضى السكر إلى تحسن فى وظائف الكبد وكان المؤشر على ذلك مستويات إنزيمات الكبدGOT, GPT حيث تواجد اقل مستوى للـ GOT بالدم (40.13) بمجموعة الفئران الضابطة السالبة. تواجدت اعلى قيمة للـ GOT في مجموعة الفئران المتغذية على اسناكس 3 بواقع (76.62).

أشارت هذه النتائج إلى أن تغذية الفئران على اسناكس الاطفال مرضى السكر المعد باستخدام المصادر النباتية سالفة الذكر اثر ايجابيا على مستوى الكوليستيرول عالي الكثافة وسلبيا على مستوى الكوليستيرول منخفض الكثافة والدهون الثلاثية كما ادى الى تحسن في وظائف الكبد.