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Pumpkin and Sunflower Seeds Attenuate Hyperglycemia and Protect Liver in Alloxan-Induced Diabetic Rats.

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ABSTRACT

This study was aimed to evaluate the hypoglycaemic, hypolipidemic and liver protective effects of pumpkin and sunflower seeds powder in male diabetic rats. A total of 24 rats were randomized into 4 groups of 6 each as follows: Group 1: Normal control; Group 2: Diabetic control; Group 3: Diabetics administered with pumpkin seeds powder (3%); Group 4: Diabetics administered with sunflower seeds powder (3%). The rats were made diabetic by alloxan (100 mg/kg body weight (BW)) injection and were treated until blood glucose reach to above 200 mg/dl. Blood samples were collected following the experiment. Liver specimens were also collected for histological analysis. Glucose, cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, ALT activity, AST activity were significantly increased, while total protein and albumin were decreased in diabetic rats as compared to the normal control group. Pumpkin and sunflower seeds significantly decreased glucose, cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, ALT activity, AST activity as compared to diabetic group. Therefore, pumpkin and sunflower seeds might be beneficial in diabetic patients.

Keywords: pumpkin seeds, sunflower seeds, hypoglycemic, blood glucose.

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INTRODUCTION

Diabetes mellitus is characterized by the lack, or relative lack of insulin. This has the effect of increasing blood glucose levels as uptake of glucose by cells is inhibited, resulting in glucose deficiency within the cells despite an abundance of glucose outside the cell (Savoca et al., 2006). Hyperglycemia is regarded as the primary cause of diabetic complications and a contributing factor to diabetic macrovascular disease especially cardiovascular disease (Soman et al., 2013).

With the number of cases expected to increase rapidly in the years to come, diabetes is a growing health challenge worldwide. With the active encouragement of the WHO, an attempt is being made to collect traditional medical information used for the treatment of diabetes for study in modern laboratories in order to scientifically evaluate therapeutic efficacies (Serraclara et al., 1998).

In diabetes, hyperglycaemia generates reactive oxygen species (ROS), which in turn cause lipid peroxidation and membrane damage and these free radicals play an important role in the production of secondary complications in diabetes mellitus (kidney, eye, blood vessel, and nerve damage) (Hunt et al., 1988). Antioxidants have been shown to prevent the destruction of β-cells (Slonim et al., 1983; Murthy et al., 1992) by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes (Halliwell and Gutteridge, 1989; Gordon, 1996).

Plants contain natural antioxidants (tannins, flavonoids, vitamins C and E, etc.) that can preserve β - cell function and prevent diabetes induced ROS formation (National Nutrition Council, 1999). In Egypt folk medicine, some traditional and edible plants have been utilized to decrease symptoms of diabetes.

Thus the aim of the present study was to evaluate the antidiabetic as well as cardioprotective role of pumpkin and sunflower seeds in diabetic animal models.

MATERIALS AND METHODS

Plant materials

Pumpkin (*Cucurbita maxima*) and Sunflower (*Helianthus annuus*) seeds were collected from local market and identified by Horticulture department, Faculty of Agriculture, Menofia University, Egypt.

Determination of total phenolic compounds

The amounts of phenolic compounds in different extracts of clove were determined with Folin-Ciocalteu reagent using the method of **(**Spanos and Wrolstad 1990**)**. 2.5 ml of 10% Folin Ciocalteu reagent and 2 ml of Na2CO3 (2% w/v) was added to 0.5 ml of each sample of plant extract solution (1 mg/ml). The resulting mixture was incubated at 45°C with shaking for 15 min. The absorbance of the samples was measured at 765 nm using UV/visible light. Results were expressed as milligrams of Gallic acid dissolved in distilled water.

Estimation of total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination according to **(**Aiyegoro and Okoh 2010**)**. One milliliter (1 ml) of sample (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm with UV visible spectrophotometer. The content was determined from extrapolation of calibration curve which was made by preparing quercetin solution in distilled water. The concentration of flavonoids was expressed in terms of mg/ml.

Gas chromatography (GC) analysis of fatty acid methyl esters (FAME)

Saturated, unsaturated and total fatty acids were determined in the oil by using methyl esters boron trifluoride method (A.O.A.C., 2000). FAME were identified on a Agilent Technologies 7890A GC equipped with flame ionization detector (PE Auto System XL) with auto sampler and Ezchrom integration system. Carrier gas

(He); ca. 25 Psi – air 450 ml/min – Hydrogen 45 ml – split 100 ml/min. Oven temperature 200ºC injector and detector 250ºC.

Design of the animal experiment

The work was carried out at the Biochemistry Department, Faculty of Agriculture, Menofia University (Egypt). To study the effect of the Pumpkin and sunflower seeds on lipid profiles and liver functions of albino rats, twenty-four male albino rats (weighting between 120 and 140 g) were used for this investigation. The rats were obtained from The Research Institute of Ophthalmology (Giza, Egypt). The rats were fed *ad libitum* on a basal diet (BD) and water for 15 days as an adaptation period. They were housed individually in stainless steel cages and divided into four groups (A,B,C,D) of six animals. The first was the normal group where the rats received a balanced diet throughout the study period (group A); all other remaining groups were treated with alloxan (100 mg/Kg body weight) to induce hyberglycemia through the feeding period. After blood glucose reached to above 200 mg/dl, we started the study as follow:

- One of each experiment continued feeding diet without any supplementation saved as hyperglycemic group (B)
- The other two groups of each experiment were allowed to feed diet with 3% pumpkin seeds as group (C) and 3% sunflower seeds as group (D).

Their food intake was monitored daily and all the rats fasted before blood sampling. The blood samples were drawn from eye plexuses after 30 days. The rats were anesthetized using diethyl ether. The weight gain of the rats was recorded weekly.

Blood sampling and analysis

Blood samples were collected after 30 day in tubes contains heparin as an anticoagulant from the eye plexuses under diethyl ether anesthesia and then centrifuged at 3000 rpm for 20 min. to obtain plasma, which was kept frozen until analysis. Glucose was determined colorimetrically by method described by Trinder (1969). The total cholesterol was analyzed calorimetrically according to Richmond, (1973) method**.** HDL - cholesterol was determined according to Lopez et al. (1977) method**.** Acording to kikuchi et al. (1998) LDL – cholesterol was calculated which LDL cholesterol = total cholesterol – (HDL cholesterol + triglycerides /5).

The triglycerides were analyzed according to Fossati and Prencipe (1982) method. Alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activities were measured according to the method described by Reitman and Frankel (1957). Total protein was determined according to Weichselbaum (1946) method ., albumin was determined according to Doumas et al. (1971) method.

Histopathological examination

Tissue specimens from liver was collected from all experimental groups at the end of experiment and fixed in 10% neutral buffered formalin, dehydrated in ascending concentration of ethanol and cleared in xylene. The fixed tissue was embedded in paraffin wax and sectioned into 4-5 µm thick, then stained with hematoxylin and eosin (H&E) method (Bancroft et *al.,*1996) . Then the sections were examined under light microscopy at 400 X magnification (DP72, Olympus).

Statistical analysis

The results of the animal experiments were expressed as the mean ± SD and they were analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p < 0.05$ was used as the criterion of statistical significance.

RESULTS

Total phenolic and total flavonoids content in pumpkin and sunflower seeds

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Data in Table (1) showed that total phenolic contents of pumpkin and sunflower seeds were (32.61 and 49.43 mg gallic acid equivalent/100g respectively). While the total flavonoids of pumpkin and sunflower were (19.7 and 28.57 mg quercetin equivalent/100g respectively).

Table (1): Total phenolic compounds and total flavonoids in pumpkin and sunflower seeds.

Plants	Total phenolic (mg/100g)	Flavonoids (mg/100g)
Pumpkin	32.61	19.7
Sunflower	49.43	28.57

Fatty acid profile of pumpkin and sunflower oils

The fatty acid composition of pumpkin and sunflower oils is presented in Table 2. According to the result shown, five fatty acids in two oils were identified, while the analysis of FAME gave the proportion of linoleic, oleic as the major fatty acids. Linoleic acid was the major fatty acid in two oils, its ratio in pumpkin was (43%) while in sunflower oil was (61.68%); the next important fatty acid in two oils was oleic acid which make (35%) in pumpkin and (27%) in sunflower.

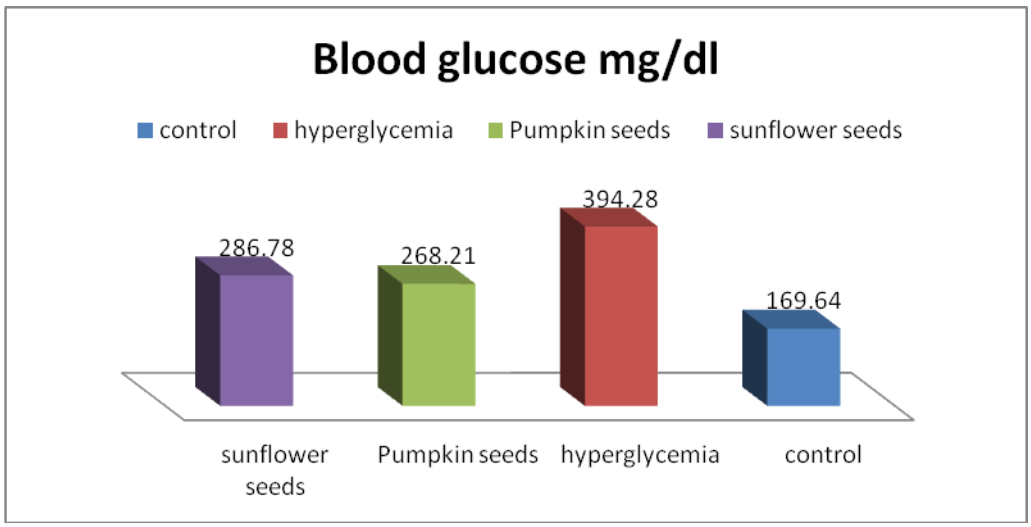
Table (2): Levels of fatty acids (%) in pumpkin and sunflower oils

Fatty acid	Pumpkin oil	Sunflower oil
Palmitic acid (C16:0)	13	5.93
Stearic acid (C18:0)	7	3.31
Oleic acid (C18:1 ω9)	35	27
Linoleic acid (C18:2 ω6)	43	61.68
Arachidic acid (C20:0)	0.5	0.23

Effect of pumpkin and sunflower seeds on plasma glucose level in hyperglycemic rats

Data in Figure (1) showed the amount of glucose in plasma by mg/dl in all treated groups compared with control , so we can notice that hyperglycemic group have the highest values (394.28 mg/dl) while the control group showed the lowest values (169.64 mg/dl), but both pumpkin and sunflower seeds groups (groups C and D) had a significant decreased in plasma glucose amount compared with hyperglycemic group (268.21 and 286.78 mg/dl respectively).

Figure (1): Effect of supplementation with pumpkin and sunflower seeds on plasma glucose level in hyperglycemic rats



Effect of pumpkin and sunflower seeds on lipid profile in plasma of hyperglycemic rats:

Data in Table (3) showed lipid profile parameters in plasma (Triglycerides, Total cholesterol, HDL-cholesterol and LDL-cholesterol) in all groups, so we can notice that hyperglycemic group have the highest

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values in all parameters: triglycerides (268.98 mg/dl), total cholesterol (249.56 mg/dl), HDL-cholesterol (62.27 mg/dl) and LDL-cholesterol (187.29 mg/dl) while the control group showed the lowest values: triglycerides (168.51 mg/dl), total cholesterol (160.77 mg/dl), HDL-cholesterol (40.6 mg/dl) and LDL-cholesterol (120.163mg/dl).

On the other hand we found that pumpkin seeds group decreased all lipid profile parameters significantly compared with hyperglycemic group, and also the same thing happened in sunflower seeds group but the effect of pumpkin seeds was better than the effect of sunflower seeds (pumpkin seeds group was high significant compared with sunflower seeds group in all parameters).

Table (3): Effect of supplementation with pumpkin and sunflower seeds on lipid profile in plasma of hyperglycemic rats

Groups	Triglycerides	Total cholesterol	HDL-cholesterol	LDL-cholesterol
Control	168.51 ± 1.77 d	160.77 ± 1.45 d	40.6 ± 4.77 d	120.163 ± 9.4 d
Hyperglycemia	268.98 ± 3.56 a	249.56 ± 4.99 a	62.27 ± 3.88 a	187.29 ± 4.7 a
Pumpkin seeds	212.03 ± 4.55 c	199.13 ± 6.23 c	46.88 ± 5.98 c	152.25 ± 4.78 c
Sunflower seeds	231.94 ± 6.78 b	217.67 ± 5.47 b	52.725 ± 6.59 b	164.94 ± 8.23 b

Valus represent means ± S.E obtained from 6 rats

Means in the same column followed by the same letters do not differ significantly, and when the means followed by different letters differ significantly at ($p \geq 0.05$).

Effect of pumpkin and sunflower seeds on liver functions in plasma of hyperglycemic rats:

Data in Figure (2) showed parameters of liver functions (total protein and albumin) in all groups, while data in Figure (3) showed the activities of ALT and AST enzymes.

The exposure of rats to alloxan led to liver dysfunctions as indicated by albumin and total protein contents (decrease in the level of albumin and total protein)and AST and ALT activities (increase in the activity of ALT and AST). Treatment pumpkim and sunflower seeds significantly protected against hepatic dysfunctions induced by alloxan treatment.

Figure (2): Effect of supplementation with pumpkin and sunflower seeds on plasma total protein and albumin levels in hyperglycemic rats

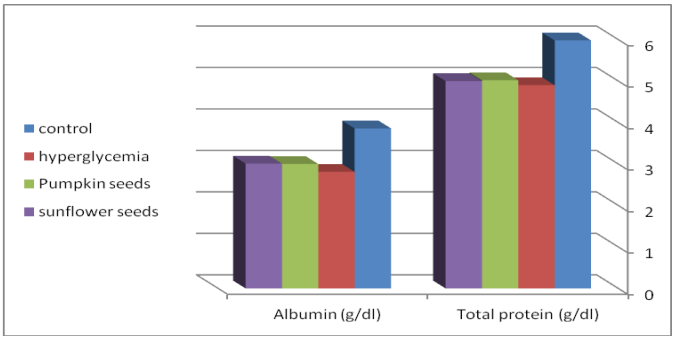
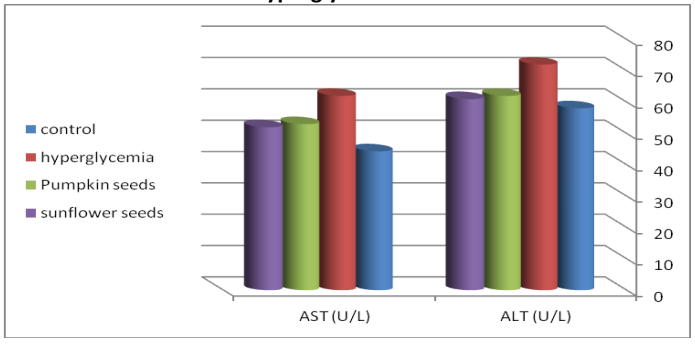


Figure (3): Effect of supplementation with pumpkin and sunflower seeds on plasma ALT and AST activities in hyperglycemic rats



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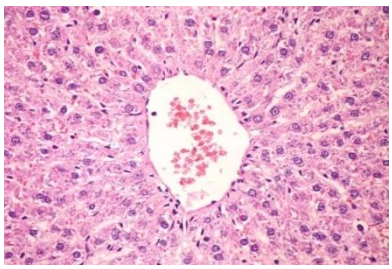
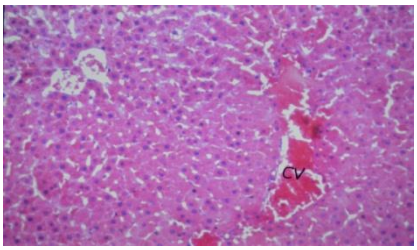
Histopathological examination

There was no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes were recorded in control groupe (Fig.1). For hyperglycemic group congestion was noticed in the central vein (Fig.2) , associated with fatty change in some few hepatocytes as well as apoptosis in others (Fig.3). The portal area showed oedema and dilatation in the portal vein (Fig.4), as well as inflammatory cells infiltration (Fig.5). We noticed dilatation in the central vein (Fig.6) in pumpkin group. As well as dilatation was detected in the central vein associated with focal pigmentation between the hepatocytes (Fig.7&8) for sunflower group.

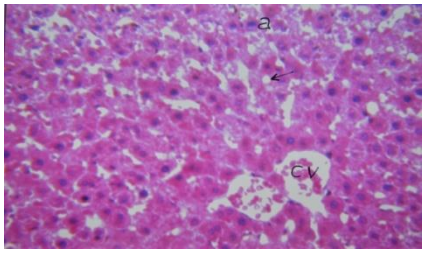
The severity of the histopathological alteration in the hepatic tissue of different experimental groups as shown in Table (4)



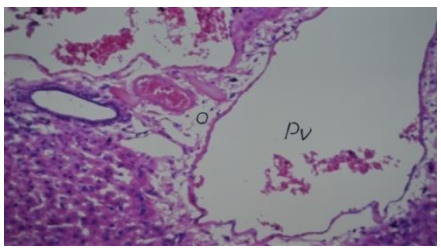
(Fig. 1) control group



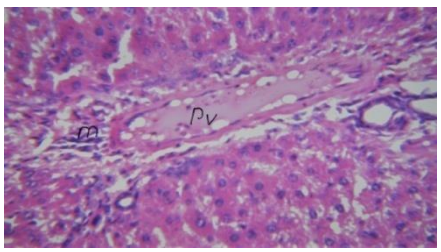
(Fig. 2)



(Fig. 3)

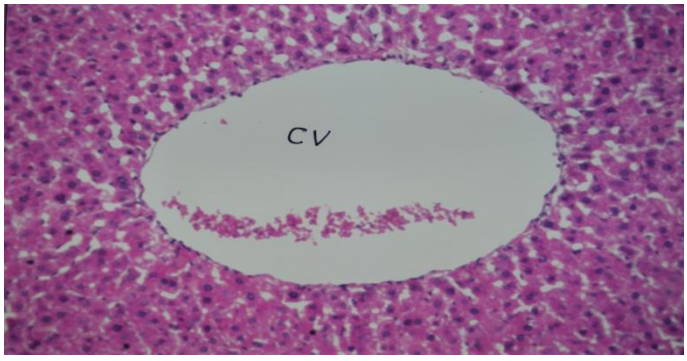


(Fig. 4)



(Fig. 5)

(Fig. 2 ,3 ,4 , 5 Hyperglycemic group)



(Fig. 6 Pumpkin group)

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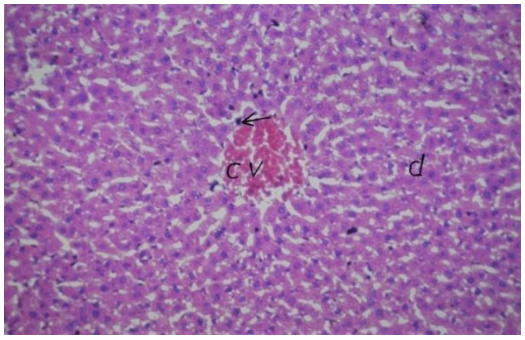

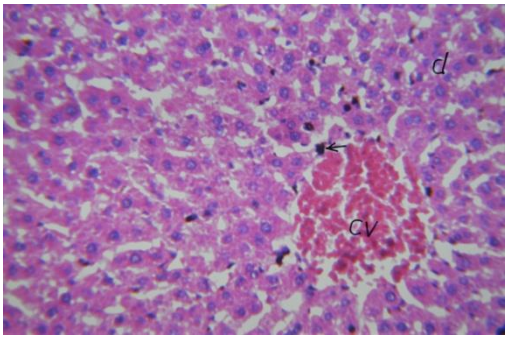

(Fig. 7, 8 Sunflower group)

Table (4) : The severity of the histopathological alteration in the hepatic tissue of different experimental groups.

Group No Histopathology. Alterations	Control	Hyperglycemic	Pumpkin	Sunflower
Congestion in B.VS	-	++	+++	++
Apoptosis in hepatocytes	-	++	-	-
Degenerative change in hepatocytes	-	+	-	-
edema in Portal area with infl. Cells infiltration	-	++	-	-
Focal cellular Pigmentation	-	-	-	+

+++ Sever 75%
++ Moderate 50%
+ Mild 25%
- Nil zero

DISCUSSION

Total phenolic and total flavonoids content in pumpkin and sunflower seeds

In general polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources and they have been shown to possess significant antioxidant activities(Van Acker et. al., 1996). Studies have shown that increasing levels of flavonoids in the diet could decrease the occurrence of certain human diseases (Hertog et. al., 1993). From our data we can noticed the high contents of both total phenolic compounds and total flavonoids in sunflower seeds compared with pumpkin seeds (total phenolic compounds 49.43 vs. 32.61 mg/100g and total flavonoids 28.57 vs. 19.7 mg/100g) , these results are in line with Kosinska and Karamac, (2006) who found that both sunflower and pumpkin seeds high content of phenolic compounds and flavonoids but also phenolic compounds and total flavonoids in sunflower seeds were higher than which found in pumpkin seeds.

Fatty acids contents in pumpkin and sunflower oils:

The analysis of FAME in pumpkin and sunflower (Table 2) gave the proportions of linoleic, oleic as the major fatty acids, together comprising more than 80% of the total identified FAME. In sunflower the major fatty acid was linoleic acid (61.68%) followed by oleic acid (27%) and these data are in line with Sadoudi et al. (2013). In pumpkin oil the major fatty acid was linoleic acid (43%) followed by oleic acid (35%) which is very close to the results of El-Adawy and Taha (2001), and Ramadan et al., (2011). The benefits of unsaturated fatty acids in reducing cholesterol levels were reported by Binkoski *et al.,* (2005) who explained the role of linoleic acid and oleic acid and it's balance when selecting food sources to replace saturated fatty acids in the diet. Thus, it can be concluded that high levels of linoleic and oleic acids in pumpkin and sunflower oils may give high nutritional values for these plant oils.

Impact of pumpkin and sunflower seeds on plasma glucose level in hyperglycemic rats:

In Figure (1) hyperglycemic group showed high significant increases in plasma glucose compared with control group. The diabetogenic effects of alloxan are partly attributed to the specific cytotoxic action mediated by reactive oxygen species generation leading to the damage of large number of β-cells accompanied by a decrease in endogenous insulin release. However, alloxan-administered rats became hyperglycaemic in a short period of time, followed by a hepatic glucose overproduction (Martinez and Milagro, 2000). On the other hand, pumpkin and sunflower seeds groups showed lower levels of blood glucose which are in line with Sedigheh et al., (2011), Youshinari et al., (2009), Sebbagh et al., (2009) who reported that pumpkin and sunflower decreased levels of blood glucose. Different mechanism of actions of anti-diabetic plants have been proposed such as potentiation of insulin effect either by increasing the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin,(Pari and Amarnath 2004) inhibition of hepatic glucose production,(Eddouks et al., 2003) inhibition of intestinal glucose absorption,(Youn et al., 2004) or correction of insulin resistance(Hu et al., 2003). These plant seeds may have exerted their antidiabetic effects by utilizing one or more of the above mechanisms.

Secondary metabolites have been reported to be involved in anti-diabetic activity of many plants (Kako et al., 1997). Our data shown that pumpkin and sunflower seeds have good amounts of total phenolic compounds and flavonoids. Fatty acids are clearly identified as insulin-secretion modulators, depending on their chain length and degree of saturation (Poitout and Robertson, 2008). Thus, linoleic acid - the major fatty acid in pumpkin and sunflower fats - may be involved in the modulation of pancreatic β-cell function, as recently reported by Feng et. al., (2006)

Impact of pumpkin and sunflower seeds on lipid profile in plasma of hyperglycemic rats:

We firstly found that alloxan administration increased triglycerides, cholesterol, HDL – cholesterol and LDL-cholesterol levels (Table 3). However, in the alloxan-induced diabetes mellitus, the rise in blood glucose is also accompanied by an increase in plasma cholesterol, triglycerides (Cam et al., 1993; Pari and Saravanan, 2002). In diabetic status, lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia and hypertriglyceridemia. This is in agreement with the fact that the glycemia level is the major determinant of total and very low-density lipoprotein cholesterol concentrations(Laakso, 1995). On the other hand, pumpkin and sunflower seeds groups showed lower levels of lipid profile parameters which are in line with Ramadan et al., (2011) who found that supplementation diets with pumpkin and apricot oils decreased levels of triglycerides, total cholesterol and LDL-cholesterol.

From our result linoleic acid and oleic acid were the majors fatty acids in both oils, and the cholesterol-lowering effect of linoleic acid (main fatty acid in pumpkin and sunflower oils) is well established from human trials. In a meta-analysis of 60 feeding studies including 1672 volunteers, the substitution of PUFA (largely omega-6, varying from 0.6% to 28.8% energy) for carbohydrates had more favorable effects on the ratio of total to HDL-C than any class of fatty acids (Mensink *et al*., 2003). Epidemiologically, the replacement of 10% of calories from saturated fatty acids with omega-6 PUFA is associated with an 18 mg/dl decrease in LDL-C which was even greater than that observed with similar replacement with carbohydrates (Mensink and Katan, 1992). Both pumpkin and sunflower seeds are oily seeds and have good amount of phytosterols and phenolic compounds. Phytosterols have been reported to exert hypocholesterolemic effect by inhibiting cholesterol absorption (Ostlund et al., 2002). Studies also point out an independent effect of phenolics improving plasma lipid profiles (Covas et al., 2006; Zern and Fernandez, 2005).

Impact of pumpkin and sunflower seeds on liver functions in plasma of hyperglycemic rats:

Alloxan administration produced diabetes status by destruction of pancreatic β-cells (Szkudelski, 2001) with changes in metabolic variables as well as kidney and liver functions. The increase of plasma AST and ALT activities indicated that diabetes may induce hepatic dysfunction as supported by previously findings showing a necrotic liver (Whitehead et al., 1999). Therefore, the increase of transaminase activities in plasma may be mainly due to the leakage of these enzymes from the liver (Larcan et al., 1979). On the other hand, treatment of the alloxan-diabetic rats with pumpkin and sunflower seeds restored the transaminase activities, this effect may be happened because of high values of flavonoids and phenolic compounds which protect liver cell from damage.

Conclusions

Pumpkin and sunflower seeds afforded substantial protection to hyperglycemic disorders and these effects are mainly mediated by minor components (total phenolic and flavoinids) as well as by unsaturated fatty acids. Further research, including food nutrition studies are needed to elucidate the human ability to use this seeds in diets.

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