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LIPID METABOLISM IN DIABETIC RATS AS AFFECTED BY CANOLA AND MUSTARD SEED SPROUTS

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ABSTRACT

Canola (*Brassica juncea L.*) and mustard (*Sinapis alba L.*) seed sprout effects on diabetic rats have no available information and to clarify their effects, both sprouts were investigated in streptozotocin (STZ) induced diabetic and normal rats. Rats were fed on a semi-modified diet containing 10% of canola or mustard sprouted using tap or saline water for sprouting ad-libitum for 6 weeks. STZ showed increases in blood sugar, low density lipoprotein cholesterol (LDL-c), vary low density lipoprotein cholesterol (VLDL-c) and triglycerides (TG). The addition of canola and mustard with or without salinty at 10% to diabetic rats diet as semi-modified diet resulted a significant decrease in blood glucose, TG and VLDL-c and data was more pronounced using mustard sprouted or saline water without changes in the HDL-c parameter. These results showed that canola and mustard especially mustard sprouted in saline water had a hypoglycemic activity in diabetic rats and partly improved lipid metabolism in the experimental rats, with non-toxic to rats in doses given over 6 weeks period in this study.

Keywords: Lipid metabolism, Canola, Mustard, Sprouts, Streptozotocin, Diabetic

INTRODUCTION

When a person has high blood glucose, either because pancreas cells do not respond to the insulin that is produced, or because the pancreas does not produce enough insulin, or its a group of metabolic diseases called diabetes mellitus as define by **Rani and Kumar (2014).** A single dose of STZ of 60 mg/kg body weight. intraperitoneally into rats results in hyperglycaemia within 72 hours **(George et al 2010 and Tahmasebpourl et al 2013).**

Streptozotocin induces one type of diabetes which is similar to diabetes mellitus with non-ketosis hyperglycemia in some animal species **(Gajdosik et al 1999 and Akbarzadeh et al 2007)**.

The role of insulin in carbohydrate metabolism is to stimulate the disposal of ingested glucose into skeletal muscle and adipose tissue and to reduce hepatic glucose production by decreasing gluconeogenesis and glycogenolysis **(Bessesen, 2001)**. Insulin suppresses the release of free fatty acids from adipose tissue by inhibiting lipolysis in lipid metabolism **(Bessesen, 2001)**.

Al-Logmanli and Zari (2009) and Aly Tahany et al (2015) found that STZ- induced diabetic rats showed significant increases in the levels of blood sugar, cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and triglycerides (TG), while body weight, was decreased compared to normal rats.

Beneficial effects on diabetes mellitus have been reported using natural products. For instance, because of the increase of insulin sensitivity caused by green tea antioxidant (polyphenol), green tea has been reported to decrease the plasma levels of blood glucose and insulin in rats **(Wu et al 2004)**. Also vitamin E was reported to improve insulin sensitivity in rats fed a high fructose diet **(Faure et al 1997).**

In the end of passed century, the attention of experts dealing with the healthy nutrition turned more and more towards the determination of the biological value of the nutritional sprouts **(Penas et al 2008).**

Dietary broccoli sprout at 10 g per day (4 weeks) improved insulin resistance among type II diabetic human patients also significantly improved blood lipid profile, decreased blood serum triglycerides and increased HDL-c concentration among human patients **(Bahadoran et al 2012).** White radish sprout improved plasma metabolites and lipid metabolism **(Hashimoto et al 2006, Taniguchi et al 2006 and Aly Tahny et al 2015)**.

Chickpea sprouts improved lipid metabolism **(Mao et al 2008)**. Five weeks dietary intake of mung bean sprout lowered blood glucose, cholesterol and triglyccride **(Yao et al 2008).** Wheat sprout is therapeutic for diabetes mellitus because wheat sprouts stimulate insulin secretion **(Lee et al 2012)**.

All lipid parameters expect HDL-c significantly elevated in diabetic rat as compared to normal control rat as reported by **Ravi et al (2005), Al-Logmani and Zari (2009), Alnahdi et al (2012) and Ahmed et al (2014).**

The present study investigated the influence of canola and mustard seed spurouts with and without salin water as semi-modified diet on blood sugar and lipid metabolites in normal and STZ-induced diabetic rats.

MATERIALS AND METHODS

Materials

Seeds of canola and mustard cv.Balady were obtained from private seed market. Canola and mustard belong to Cruciferous family. The seeds were cleaned from all impurities for sprouting using jar method described by **Abdallah** (**2008)**. The harvest sprouts (3 days old for canola and 6 days for mustard for both germinated in tap water "TWS" and in 1000 ppm NaCl solution "SWS"), were washed and sun-air dried for 3 day according to **Dzowela et al (1995)** before crushed into powder for rat diet. Streptozotocin (STZ) was obtained from Biomedicals.

Experimental animals and diabetes

This study has been done using 36 male Albino Spargue-Dawely rats with a mean weight of 158 ± 18 g. The rats were provided by the animal house of the Egyptian Organization of Biological Products and Vaccines, Egypt. They were raised in the animal house of Biology Laboratory, Faculty of Agricultural, Cairo University, Giza, Egypt. The animals were divided into six equal groups of 6 rats; each and housed individually in stainless steel cages with wire mesh bottoms and maintained at 25 ± 2 °C, relative humidity of 50-60% and 12/12 h light/dark cycle throughout the experiment for a week for laboratory acclimatization. Five groups of the rats were injected intraperitoneally with STZ (60 mg / kg body weight) to induce a diabetic status according to **George et al (2010), Tahmasebpourl et al (2013) and Aly Tahany et al (2015).**

The experimental design

The six groups of rats were detalied as follows:

G1 = Normal health control rats fed on astandard diet.

G2= Diabetic control rats fed on astandard diet.

G3= Diabetic rats fed on SWS canola semi-modified diet.

G4- Diabetic rats fed on TWS canola semi-modified diet.

G5= Diabetic rats fed on SWS mustard semi-modified diet.

G6- Diabetic rats fed on TWS mustard semi-modified diet.

Standard (AIN-76 diet) prepared for G1 and G2 groups and fed on semi-modified diets (AIN-76 diet) containing 10% of SWS and TWS canola prepared for G3 and G4 respectively or 10% SWS and TWS mstard prepared for G5 and G6, respectively. at the expense of corn starch

ad libitum for 6 weeks. observing body weights. The composition of normal standard diets (AIN-76 diet) contained the following component: corn starch mix 65%, casein 20%, cellulose powder 5%, corn oil 5%, AIN-76 mineral mix 3.5%, AIN-76 vitamin mix 1.0%,methionine 0.3% and choline chloride 0.2%.

Blood sample collection and glucose determination

Six blood samples were collected from rats of each group from the retero oribital plexus of veins according to **Shermer (1967)** after six week in clean dry sterile and labeled centrifuge tubes.Rats were fasted for 12 h, and then slightly anaesthetized with carbon dioxide gas. Separating serum was done by centrifugation at 1500 r.p.m for 5 min. Blood sugar was determined enzymatic colorimetrically in the serum according to the method of **Trinder (1969)**.

Analytical Methods

The total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) and serum levels triglycerides (TG) were measured with an automatic anolyzer using diognostic kit for eash according to **Fossati et al (1982), Allain et al (1974), Wieland and Seidel (1983) and Burstein et al (1980)** respectively**.** Very low-density lipoprotein cholesterol (VLDL-c) by subdivision (TG\ 5).

Statistical analysis

Obtained data were statistically analyzed according to the method of **Snedecor and Cochran (1980)** and LSD ($P < 0.05$) was used to compare the significant difference between mean of treatments.

RESULTS AND DISCUSSION

1- Effect of sprouting using saline water on canola and mustard sprout characters

Data in **Table (1)** showed fresh weight of canola and mustard sprout ratio about 1:6.9 and about 1:3.3 per equal fresh weight of canola and mustard seed, respectively which obtained by using jar method for 3 day old canola and 6 day old mustard sprout.

However canola ratio (> 1:5) was obtained before on clover sprouts by **Sawyer et al (1985) and Abdallah (2008).** While, mustard recorded lower sprout ratio. However, the sprout ratio increased with sprouting using allow NaCl concentration compared with using tap water for both canola z. mustard. The sprout average length was 4.02 cm for canola sprout and about 2.93 cm for mustard sprout using tap water for sprouting. Using Na Cl at 1000 ppm recorded the tallest canola sprout length (5.38 cm) while 2000 ppm recorded the tallest mustard sprout length (3.52 cm). The increment in the sprout ratio and length regarded to the higher fresh weight of sprout, since the sprout had greater water content than the original on a fresh – weight basis.

Table 1. Effect of NaCl concentrations in sprouting solution on 3 day old etiolated canola and 6 day mustard sprouts characters. (Combined of two experiments)

Character / NaCl Concentration	Sprout radical length (cm)	Sprout hypocotyl length (cm)	Sprout length (cm)	10 sprouts fresh weight (mg)	10 sprouts dry weight (mg)
	Canola	**3 day old**			
Control (Tap water)	**4.02 bc**	**2.083a**	**1.864a**	**0.210a**	**0.041a**
1000 ppm	**5.38 a**	**3.113a**	**2.273a**	**0.237a**	**0.023a**
2000 ppm	**4.34 b**	**2.425a**	**1.873a**	**0.207a**	**0.017a**
3000 ppm	**3.72 c**	**2.11 b**	**1.40 c**	**0.21 b**	**0.020 ab**
LSD 0.05	**0.509**	**0.355**	**0.371**	**0.025**	**0.003**
	Mustard	**6 day old**			
Control (Tap water)	**2.93 c**	**2.08 c**	**0.82 c**	**0.17 c**	**0.039 a**
1000 ppm	**3.32 b**	**2.29 b**	**0.96 b**	**0.22 ab**	**0.041 b**
2000 ppm	**3.52 a**	**2.58 a**	**1.10 a**	**0.24 a**	**0.048 b**
3000 ppm	**3.34 b**	**2.42 ab**	**0.98 b**	**0.21 b**	**0.040 b**
LSD 0.05	**0.173**	**0.189**	**0.098**	**0.018**	**0.0684**

Means in each column for followed by the same letter are not significantly different at the 5% level

Canola and mustard dry weight decreased as NaCl concentration increased. The dry control mustard sprout recorded the highest percentage loss in sprout dry matter (about 25%) compared with loss in control canola sprout dry matter (about 4.3) **(Table 1).** The more percentage loss in control mustard sprout than in canola sprout may be due to the hydroponic sprout production for 6 day for mustard sprouts comparsed to 3 day sprouting for canola sprouts which may loss less during respiration period. The decreased dry weights during sprouting results are in agreement with those reported by **Chavan and Kadam (1989), Wanasudara et al (1999) and Anwar et al (2015)**

2- Effect of canola and mustard sprouts on experimental rats.

A- Effect of semi-modified diets on blood glucose

Concerning blood glucose levels measured at the end of the experiment (6 weeks after feed treatment), data in **Table (2)** showed hyperglycemia with STZ diabetic control and treated groups by mustard sprout (groups 5 and 6). The hyperglycemia with STZ diabetic control was observed during the whole 6 weeks (studied period) with blood glucose increment which amounted 283.4 mg/dl at last week of the experimental period. The significant increases in blood glucose level in STZ-diabetic are in agreement with **Bukan et al (2003), Amer et al (2004), Akbarzadeh et al (2007) and El-Abd et al (2007) and Aly Tahany et al (2015).**

Feeding diabetic rats on canola or mustard semi-modified diets for 6 weeks studied period decreased the hyperglycemia but it was more than normal state compared with normal treatments. The decreased in blood glucose levels recorded about 122.5 and 128.7 mg/dl in STZ induced diabetic rats treated with the diets containing SWS canola (NaCl 1000 ppm) and TWS canola respectively. The diabetic control significantly showed about 283.39 mg/dl at the same time. On the other hand, mustard sprouts semi-modified diets decreased blood glucose levels to about 180.5 and 165.2 mg/dl in both SWS mustard (NaCl 2000 ppm) and TWS mustard, respectively with non significant with both negative or positive control.

In the present study, canola semi-modified diets significantly and gradually reduced blood glucose levels in STZ- diabetic rats during the 6 weeks of treatments which also demonstrated that there was a significant high rate of glucose disposal. Similar results were also obtained by **Taniguchi et al (2006 and 2007) and Aly Tahany et al (2015)** with Japanese and Egyptian radish sprouts and clover sprouts. However, the mechanism of these sprout which decreased blood glucose level, has not been clearly defined. Previous studies demonstrated that radish sprouts and radish extrct and their active constituent have proven free radical scavenging and antioxidant activates **(Sgherri et al 2003, Peluso 2006, Wang et al 2010 and Zhou et al 2013).**

The canola or mustard sprouting with tap water or saline water, semi-modified diets were effactive as hypoglycemic agents where glucose levels reduced at the end of experimental period by about 90 %.

Table 2. Effect of TWS and SWS of canola and mustard, semi-modified diets on fasting blood glucose and body weight, of experimental rats.

Rat Groups	Fasting blood glucose (FBG)		Rats body weight (g)	
	Mg/dl	%	G	%
G1-Normal control	119 b	100	241.35 bc	100
G2-Diabetic control (+STZ	283.39 a	238	220.72 c	91.5
G3- SWS canola	122.53 b	103	284.27 ab	117.8
G4- TWS canola	128.71 b	108	304.01 a	126
G5- SWS mustard	165.21 ab	139	240.29 bc	99.6
G6- TWS mustard	180.53 ab	152	233.76 bc	96.9
LSD 0.05	**126.55**		**59.782**	

Means in each column followed by the same letter are not significantly different at the 5% level

B- Effect of SWS and TWS of canola and mustard semi-modified diets rats on rats body weight

Table (2) shows mean body weight of the experimental rats, 6 weeks after STZ injection at the end of the experiment (final weight). Data showed that STZ– induced diabetic rats fed on the normal diet had the lowest body weight.

STZ– induced diabetic rats given diet containing 10% of canola sp had higher body weight than rats given normal diet either for diabetic control or normal control rats fed on normal diet at the end of experiment. Data was more pronounced with rats fed on diets containing 10% TWS canola. Previous studies showed that diabetic rats had significantly lower final-weight than control **(AL-Rawi 2007a, Al-Logmanli and Zari 2009, Alnahdi, 2012 and Tahmasebpourl et al 2013 and Aly Tahany et al 2015).**

The decrease in body weight of diabetic rats be due to catabolism of fats and protein. However, STZ– diabetic rats feeding on canola sprout especially TWS for 6 weeks showed increase in body weight which may be explained by increased insulin secretion; increased insulin- like compounds increased food consumption (**Farouque and Meredith, 2003, Amer et al 2004 and Taniguchi et al 2007).**

C- Effect of TWS and SWS of canola and mustard semi-modified diets on lipid profile of the experimental animals

Table (3) shows serum lipid profile (TC, TG, HDL-c, LDL-c and VLDL-c). Values were increased in STZ –induced diabetic control group when compared with normal control. The mustard sprout from both tap water (TWS) and saline water (SWS) semi- modified diets fed STZ- diabetic rats showed re- adjustment for TG, VLDL-c levels relative to control group. Percentage of recovery in the serum level of TG or VLDL-c was about 47.7 % and 29.6 % by feeding on TWS and SWS mustard semi-modified diets, respectively in respect to diabetic control. However, TWS canola semi-modified diets recover TG and VLDL-c by about 34.4% but LDL-c increased by about 143.8% in respect to diabetic control.

On the other hand, SWS canola semi-modified diets showed no significant effect on TG, VLDL-c and LDL-c. Moreover, HDL-c level showed no significant changes between groups **(Table 3).** However, the results of increasing levels of serum TG and VLDL-c in STZ-induced diabetic rats are in agreement with those abtained by **Ravi et al (2005), Al-Logmanli and Zari (2009) and Alnahdi (2012).**

The abonormal high concentration of serum lipid constituents in diabetic animals are due mainly to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibitis the hormone-sensitive lipase as reported by **Pushparaj et al (2000)**. Diabetic rats converted fatty acids in serum into phospholipids and cholesterol in the liver. These two substances along with excess triglyceides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins **(Bopanna et al 1997)**. The present study showed that TWS, SWS mustard and TWS canola had favorably modified serum lipid profile in rats with decrease in triglyceride (TG) and VLDL-c.

Table 3. Effect of TWS and SWS of canola and mustaed semi-modified diets on lipid profile of the different experimental rats.

Groups/Parameters	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
G1.Normal control	70.101 [c]	59.32 [c]	48.63 [a]	20.04 [b]	11.86 [c]
G2.Diabetic control (+STZ)	75.22 [c]	154.09 [a]	61.49 [a]	19.46 [b]	30.82 [a]
G3. SWS canola	90.36 [b]	130.84 [ab]	46.31 [a]	21.01 [b]	26.17 [ab]
G4.TWS canola	110.24 [a]	101.09 [abc]	42.57 [a]	47.45 [a]	20.22 [abc]
G5.SWS mustard	95.24 [b]	80.52 [bc]	57.81 [a]	29.31 [b]	16.11 [bc]
G6.SWS mustard	98.02 [ab]	108.55 [abc]	60.15 [a]	23.18 [b]	21.71 [abc]
LSD 0.05	14.547	60.184	N.S	11.3	12.038

N.S= Not significant.

Values in the same column with the same letter are not significant at $P \leq 0.05$.

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تأثير نبت بذور الكانولا والخردل على التمثيل الغذائي للدهون في الجرذان المصابه بداء السكري [60]

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تم استخدام بذور الكانولا والخردل في انتاج النبت بعمر 3 أيام للكانولا و6 أيام للخردل باستخدام مياه الصنبور أو المياه المملحة بكلوريد الصوديوم، وتم تسجيل النتائج المتحصل عليها من حيث صفات كلا النوعين، واستخدم نبت بذور الكانولا والخردل لدراسه تأثيرهما علي خصائص السكر في دم الجرذان المصابة بالسكري وتم توزيع الجرذان الى 6 مجموعات: الاولى المرجعية القياسية السليمة مع العليقة القياسية العادية، والثانيه المرجعيه القياسيه المصابة بداء السكري مع العليقة القياسية العادية، والثالثة المصابة بداء السكري مع النظام الغذائي المعدل بنبت الكانولا الناتج بأستخدام الماء المملح بكلوريد الصوديوم بتركيز 1000 جزء في المليون، والرابعه المصابه بداء السكري مع نفس النظام الغذائي المعدل لنبت الكانولا الناتج بإستخدام ماء الصنبور، والخامسه المصابه بداء السكري مع النظام الغذائي المعدل بنبت الخردل الناتج بإستخدام الماء المملح بكلوريد الصوديوم بتركيز 2000 جزء في المليون، والسادسه المصابه بداء السكري مع نفس النظام الغذائي المعدل لنبت الخردل الناتج بإستخدام ماء الصنبور، وكل مجموعه إشتملت على 6 جرذان وتم أحداث داء السكري بواسطه الحقن بالاستربتوزوتوسين (60 مج/ كجم من وزن الفأر) .

وأظهرت الجرذان المصابه بداء السكري نتيجة الحقن بالاستربتوزوتوسين زيادة معنويه في مستوى جلوكوز الدم والدهون الثلاثيه والليبوبرتين منخفض الكثافه (LDL–c) والمنخفض الكثافه جدا (VLDL–c) وأضافه نبت الكانولا والخردل الناتج بأستخدام ماء الصنبور أو الماء المملح بمعدل 10 % الي عليقه الجرذان المصابه بداء السكري كنظام غذائي معدل، أدت لانخفاض جلوكوز الدم، وان الزيادة أو النقص الحادث بواسطة داء السكري بسبب الحقن بالاستربتوزوتوسين قد تم تحسنها بواسطه النظام الغذائي المعدل بنبت الكانولا والخردل، ومن هذه النتائج يمكن الوصول الى أن النظام الغذائي المعدل بنبت الكانولا والخردل أظهر القدرة على تخفيض أرتفاع السكر بالدم، وله تأثيرات مضادة للاكسدة في الحالات المصابه بداء السكري، كما أن الجرعه المستخدمه من نبت الكانولا والخردل في النظام الغذائي المعدل للجرذان تعتبر ضروريه وغير سامه لمدة 6 أسابيع هي فترة الدراسه.

الكلمات الدالة: التمثيل الغذائي، الكانولا، المستردة، النبت، الاستربتوزوتوسين، داء السكري

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