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Effect of Red Cabbage Leaves as Hypoglycemic and Antioxidant in Diabetic Rats Fed on HFT/HFR diet

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ABSTRACT

Thirty six rats were used to study the effect of extract of red cabbage against diabetes induced by HFT/HFR diet. Rats were randomly distributed into 6 groups each containing (n=6). The first group kept as normal control fed standard diet only. The other six groups fed on basal diet and HFT/HFR diet and reclassified into positive control, red cabbage (RC) as powder and extract at levels groups (2.5% , 5% of the weight of basal diet), (250 mg/kg and 500 mg/kg weight of the rat) . Our results showed significant increase in weight gain, food intake and food efficiency ratio in all groups treated with RC as powder and extract groups comparing with (+ve) group, as well as significant reduction in serum glucose, HbA1c, lipid profile parameters, liver and kidney biomarkers and nitric oxide (NO) levels, moreover significantly increase in insulin, HDL-c, total antioxidant capacity (TAC) and superoxide dismutase (SOD) levels in treated all groups treated with RC powder at level 5% and extract 500 mg/kg groups comparing with (+v) group. This study suggests that treatment with red cabbage powder and extract has a beneficial effect against diabetes and hyperlipidemia.

Keywords: Red cabbage, Hyperlipidemia, Toxins, Metabolic syndrome

INTRODUCTION

Read Crambe (RC) also called cabbage, is a leafy vegetable that grows in the Maghreb, southern half of Asia, and southern Europe, and it is a member of the cruciferous family (*Brassica oleracea* var. *Capitata*) (Jacqueline and Marcus, 2013). Crambe read, which belongs to the genus *Brassica*. Green, purple crambe turns blue in a variety of citrus fruits into the cooking pot to preserve its purple color. A rich source of antioxidants contains eight times the antioxidants found in green cabbage, as purple cabbage contains vitamin C, carotenoids, and flavonoids, including anthocyanins, kompherl, which are other phytochemical compounds linked to reducing the risk of cardiovascular disease (Dinkova and Kostov, 2012 and Wiczowski, *et al.*, 2013). It is an important source of vitamin A, which is absorbed in the body in several forms of Zeaxanthin and beta-carotene, and vitamin A is especially important for maintaining the health of the eyes and the nervous system. Vitamin K1 and vitamin K2 are important for maintaining healthy and strong bones. Crambe contains folic acid, which works by boosting certain enzymes which in turn helps to purify the body of toxins. And the crambe has anti-bacterial properties, as it works to treat stomach and intestinal ulcers, skin infections, tumors, rheumatism and gout. Crambe also contains two important components (sulforaphane and indole) (Veber *et al.*, 2020). As they have an effective role in preventing cancer, diabetes, heart disease, osteoporosis and hypertension.

The body needs a maximum of calories and fats in its diet, usually when we eat more than the needs; the body does not absorb it and begins to store it (Krishna, *et al.*, 2012). When our excess fat consumption is repeated, it resides in the arteries at a speed that exceeds the ability to store it, which causes it to accumulate on the walls of blood vessels, thus

leading to various heart diseases, stroke, obesity, metabolic syndrome, type 2 diabetes, high blood pressure, high triglycerides, and high abnormal blood sugar and cholesterol levels Hanaa, (2017). An elevated blood lipid level is equivalent to 200 - 499 mg / dl, while above this limit it is considered too high (Prabu and Natarajan, 2013 and Bordoloi, *et al.*, 2014). Metabolic syndrome is a condition in which high blood pressure, obesity and high blood sugar combine, which increases the risk of heart disease and low levels of thyroid hormones (hypothyroidism). This search aimed to indicate the effect of red cabbage leaves as hypoglycemic and antioxidant in diabetic rats fed on HFT/HFR diet.

MATERIALS AND METHODS

Materials:

Read Crambe (*Crambe*) leaves were obtained from local market, Mansoura, Egypt. Fructose was purchased from the International Company for Scientific and Medical Supplies, Cairo, Egypt.

Experimental rats: thirty six weanling female Sprague-Dawley rats weighing 70 ± 5 g in aged of 3 weeks were used. The rats were acclimatized for 1 week before dietary manipulation and were housed individually in cages under laboratory healthy conditions.

Methods:

Preparation of read crambe: The collected plant was rinsed water and dried at room temperature (25-30 °C) for 2 weeks. The dried plant sample was ground into cabbage powder using a mortar and pestle, according to Russo, (2001). The dried cabbage were powdered mechanically and soaked in 95% ethanol in brown bottles at room temperature (25 -30 °C) in dark place for 5 days and was shaken gently every day. The

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mixture was filtrated by suction pump in bokhnnar cone throw filter paper. The ethanolic extract was concentrated by heating under vacuum at 62°C (Irkin and Korukluoglu. 2007).

Analytical Methods

Moisture, protein, fat, fiber and ash were determined according to AOAC (2000). Carbohydrates and energy value by calculated.

Biological evaluation

Experimental Design: Two types of diets were used in this study: 1- basal diet was prepared according to (Reeves *et al.*, 1993). 2- (high fat/high fructose) HFT/HFR diet, consisted of basal diet contain 20% fat (15% beef tallow + 5% corn oil) combined with fructose added in drinking water at 13% w/v which is the range of concentration that reported for soft drinks (Light *et al.*, 2009). Basal blood glucose levels were measured in the tail vein blood using a Medisense Precision Q.I.D glucose meter (Abbott Laboratories). The rats were given 2 g/kg body weight of glucose via oral gavage as a 40% solution. Tail vein blood samples were withdrawn at 0, 30, 60, 90, and 120 min following glucose administration. After one week female rats were randomly assigned into four groups (6 rats) as follow:

- Group (1):** Normal control rats (ve-) fed on basal diet.
- Group (2):** Diabetic rats induced by fed on basal diet and HFT/HFR diet used as a positive control group (ve+).
- Group (3):** Diabetic rats fed on received (RC) as powder by 2.5% of the weight of basal diet.
- Group (4):** Diabetic rats fed on received (RC) as powder by 5% of the weight of basal diet.
- Group (5):** Diabetic rats fed on received (RC) extract by 250 mg/kg of the weight of the rat.
- Group (6):** Diabetic rats fed on received (RC) extract by 500 mg/kg of the weight of the rat.

At the end of the period (6 weeks), rats were fasted overnight and the blood samples were collected directly from portal vein into non-heparinized centrifuge tubes. Serum were separated by centrifugation and then were frozen at -20 °C for biochemical analysis.

Biochemical Parameters:

Fasting serum glucose and insulin levels was according to (Thorell and Lanner, 1973 and Matthews *et al.*, 1985) respectively. Serum TG, TC and HDL levels were determined by enzymatic method that had previously

described by (Fossati and Prencipe, 1982, Allain, 1974 and Burstein *et al.*, 1970), respectively. Serum LDL levels were calculated according to the equation of (Friedwald *et al.* 1972). Serum alanine, (ALT and AST), and ALP enzymes activity and serum total bilirubin were estimated according to (Reitman and Frankel, 1957, Kind and King, 1954 and Dumas *et al.*, 1973). Serum uric acid, creatinine and urea were determined according to the method of (Fossati *et al.*, 1980, Bartles, 1972 and Patton and Crouch, 1977). Serum total antioxidant and oxidant capacities were measured according to (Cao *et al.*, 1993 and Flohe and Gunzler, 1984), respectively.

Statistical Analysis:

The statistically analyzed were using computerized SPSS and ANOVA test using Duncan’s multiple range test and p<0.05 was used to indicate significance between different groups (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Red cabbage leaves were analyzed to obtain its chemical composition and results were illustrated in table (1). The amount of moisture, ash, protein, fiber, fat and carbohydrates of Red cabbage leaf (85.11, 2.91, 1.68, 2.7, 0.1 and 7.5 w/w) respectively. These results were agreed with the findings of (Jacqueline and Marcus , 2013) who found that, the amounts of protein, fat, ash, fiber and carbohydrates of whole crambe leaves was (1.54, 0.1, 92.18, 2.3 and 9.23 g/100g), respectively.

Table 1. Chemical composition of red cabbage

Constitutes (%)	Value(wet weight)	Value(dry weight)
Moisture	85.11	----
Ash	2.91	3.26
Protein	1.68	1.89
Fiber	2.7	3.2
Fat	0.14	0.21
Carbohydrates	7.5	91.44
Energy value (Kcal/100g)	38	---

The effect of red cabbage leaves powder and extract on food intake (FI), body weight gain (BWG) and food efficiency ratio (FER) of the experimental diabetic rat groups is presented in Table (2). The mean value of feed intake of rats fed on basal diet ((ve-) group) was 15.25 and 11.50 g/day for (+v) group.

Table 2. The effect of HFT/HFR diet on growth parameters in experimental groups

Groups Parameters	control		Protective with cabbage			
	-ve G1	+ve G2	2.5% G3	5% G4	250 mg/kg G5	500 mg/kg G6
(BWG)(g)	122.25±10.52 c	141.50±11.35 a	135.75±11.30 a	132.75±11.91 a	131.84±11.91 a	129.04±11.11 a
(FI)(g)	11.50±1.29 b	15.25±1.25 a	14.75±1.95 a	14.50±1.73 a	14.50±1.73 a	15.02±1.12 a
(FER)%	0.24±0.002 c	0.33±0.001 a	0.30±0.001 a	0.32±0.002 a	0.32±0.002 a	0.31±0.001 a

Values with the same letters indicate insignificant difference and vice versa. (BWG)=body weight gain, (FI)=food intake and (FER)=food efficiency ratio.

While (FI) for all treated diabetic rat groups was decreased than that of the (ve-) group, on the other hand, these treatments showed an increasing of the mean values of (FI) in compared to (+v) group.

There was a significant decreasing in values of FER and body weight gain in (+v) group in compared to (-v) group, while there was an improvement in those values of all treated groups in comparing to the (ve+) group. The highest increase in BWG in all treated groups recorded for the group treated

with 500mg/kg of red cabbage extract in compare to (ve+) group.

It is clearly shown that high fat diet and high fructose diet have a bad effect on experimental rats specially in affecting the food intake and FER. Those results were agreed with (Anna *et al.*, 2012) who stated that food rich in fats produce oxidative stress which harm the body. high-fat meal can release a substance called endotoxin, this substance was released in high amount greater than smoking three cigarettes.

Endotoxins promote hardening of arteries and leads to obesity.

Our results were matched with (Dinkova and Kostov, 2012) and this due to the presence of glucosinolates and isothiocyanates in red cabbage. Those compounds are playing an important role in human health through its powerful

Results in Table (3) representing the effect of red cabbage leaves powder and extract on serum glucose, insulin level and glycated hemoglobin of the diabetic rat groups.

The mean value of fasting blood glucose (FBG) in the first week of rats fed on basal diet ((-v) group) was 88.75 mg/dl while (+v) group was 355.25 mg/dl. In second week, FBG was 89 mg/dl for (-v) group and 89.50 mg/dl in the sixth week. The positive group showed significant increase in FBG level in second and sixth week of experiment.

There was a significant improvement in the FBG results of treated rat groups during all the experiment period especially in the sixth week in compare to the (+v) group.

The FBG in the sixth week of diabetic rats fed (RC) powder by 2.5% was 182.75 mg/dl, 168.25 mg/dl for diabetic rats fed (RC) powder by 5%, 192.25 mg/dl for diabetic rats fed (RC) extract 250mg/kg and 180.50 mg/dl for Diabetic rats fed (RC) extract by 250mg/kg.

The best result was achieved by group (4) which was fed on (RC) powder 2.5%.

The insulin level for rats fed on HFT/HFr diet was 8.60µ/ml which was less than negative group that recorded 16.05µ/ml. on other hand; the insulin level of rat groups treated with (RC) showed a significant increase in compare to

antioxidant properties. Isothiocyanates have a wide range of therapeutic advantages in fighting the oxidative stress , it also has a great function in prevention of reduction in insulin sensitivity that may happened by high fat diet (Draghici, *et al.*, 2019).

positive control group. Hb A1C% was significantly increased in (+v) group than the negative control group. Treating with (RC) powder and extract had a significant impact on Hb A1C% as there was an improvement in Hb A1C% results comparing to positive control group.

High fat and high fructose diet leading to a decrease in endogenous insulin production and long-term hyperglycemia, high fat diet stimulate nitric oxide production which it is consider as an important key in DNA alkylation and ROS production (Hanaa, 2017).

These results were in agreed with (Nabavi , *et al.*, 2019) who reported that plants containing high concentration of anthocyanins markedly increased the utilization of glucose by tissues in diabetic rats and decreasing glucose levels. Bordoloi, *et al.*, 2014 and Hanaa, (2017) found that there is a potential effect of this flavonoid family in reducing other insulin resistance-related disease and hyperlipidemia. Red cabbage can reverse hyperglycemia effect by enhancing insulin secretion and decrease insulin resistance due to Anthocyanins and cyanidin-3, cyanidin-3-glycoside playing an important role in increase of insulin protein and encoding of insulin as it stimulate insulin-like growth factor II (Wiesław, *et al.*, 2016).

Table 3. Serum glucose, insulin and levels glycated hemoglobin of the experimental HFT/HFr rat groups

Groups Parameters	control	control	Treated with cabbage			
	-ve G1	+ve G2	2.5% G3	5% G4	250mg/kg G5	500mg/kg G6
(FBS) (mg/dl)1week	88.75±7.08 d	355.25±13.04 a	249.75±18.01 b	241.75±18.01 c	228.60±14.14 c	224.75±18.06 c
(FBS) (mg/dl)2week	89.00±4.08 e	398.75±10.31 a	274.00±17.02 c	226.25±18.83 d	229.55±13.96 d	219.15±16.18 d
(FBS) (mg/dl)6 week	89.50±5.91 e	411.25±18.99 a	182.75±16.04 b	168.25±16.15 d	182.25±14.50 b	172.50±15.58 d
Insulin (µ/ml)	16.05 ±1.67 a	8.60±1.16 d	14.74±2.31 c	15.55±1.99 b	15.26±1.99 b	16.03±1.65 a
(Hb A1C)%	5.72±0.71 c	12.30±1.70 a	7.57±0.68 b	5.90±0.66 c	6.85±0.54 b	6.02±0.80 b

Values with the same letters indicate insignificant difference and vice versa.

(FBS)= Serum glucose & (HbA1C)= glycated hemoglobin.

Table (4) showed the effects of red cabbage powder and extract on experimental diabetic rat groups on total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and total lipid (TL). The results showed that positive control rats had higher (P≤0.05) TC, TG, LDL, TL and VLDL than negative control group. However, the HDL value of (-v) group had an opposite trend. Treating

diabetic rats with red cabbage powder and extract caused significant (P≤0.05) reduction in TC, TG, LDL, TL and VLDL as compared with positive control group. red cabbage extract 500mg/kg was the most effective group (P≤0.05) in reducing serum TC, TG, LDL, TL and VLDL. But, these values are still higher (P≤0.05) than those values of negative control rats.

Table 4. Some serum lipids profile (mg/dl) of the experimental HFT/HFr rat groups

Groups Parameters	control	control	Treated with cabbage			
	-ve G1	+ve G2	2.5% G3	5% G4	250mg/kg G5	500mg/kg G6
(TC)mg/dl	63.80 ±9.34 d	105.55 ±12.26 a	84.62 ±11.48 b	75.57 ±10.48 c	71.56 ±5.38 c	61.61 ±5.31 d
(TG)mg/dl	95.77 ±9.49 d	148.38 ±11.61 a	122.18 ±10.73 b	94.27 ±8.55 d	97.27 ±3.35 d	92.24 ±3.35 d
(HDL-c)mg/dl	37.57 ±3.71 a	26.15 ±2.43 c	30.52 ±2.91 ab	331 ±3.43 b	34.87 ±2.87 a	35.79 ±2.11 a
(VLDL-c)mg/dl	19.15 ±1.90 c	29.68 ±2.32 a	24.54 ±2.15 b	18.97 ±1.71 c	19.45 ±0.67 c	18.06 ±0.67 c
(LDL-c)mg/dl	22.77 ±2.04 e	41.30 ±3.92 a	32.52 ±2.06 b	30.52 ±2.18 c	26.30 ±2.19 d	25.52 ±2.80 d
Total lipidmg/dl	223.92 ±11.83 e	333.32 ±12.10 a	283.28 ±14.10 b	265.15 ±13.36 c	239.12 ±6.24 d	234.15 ±6.24 d

Values with the same letters indicate insignificant difference and vice versa.

TC: Total Cholesterol. TG: Triglycerides. (HDL-c): High density lipoprotein. (LDL-C): Low density lipoprotein.

(VLDL-c): Very low density lipoprotein. (TL): Total lipid

Diabetic rats fed on red cabbage either powder or extract showed significant (P≤0.05) improvement in HDL level in compared to (+v) group (Wu and Prior, 2005) investigated the effects anthocyanin contained herbal supplements on lipid profile in patients suffering of dyslipidemia. They concluded that herbal supplements containing anthocyanin can decrease LDL-cholesterol and

improves HDL-cholesterol levels and enhances cellular cholesterol efflux to serum. Red cabbage has a potential therapeutic effect in lowering high cholesterol level in blood and lowering the risk of atherosclerosis, those effects due to presence of dicaffeoylquinic, flavonoids epicatechin, cinnamic acids and galocatechin which demonstrated a

beneficial effect against hypertriglyceridemia and hypercholesterolemia (Veber *et al.*, 2020).

Further, red cabbage's polyphenols like flavonoids and isothiocyanates as well as ascorbic acid (vit. C) which play a great role in down-regulate HMG-CoA reductase enzyme hence possess hypolipidemic potential (Amnah .2013).

Results shown in Table (5) are representing the treating effect of red cabbage on diabetic rat's liver and kidney function profile. (-v) group had lower ($P \leq 0.05$) AST, ALT, creatinin, uric acid and urea and urea activities than positive control group. Supplementation diet of rats with red cabbage significantly ($P \leq 0.05$) reduced AST, ALT, creatinin, uric acid and urea compared with positive control rats. red cabbage

extract 500mg/kg was the most effective group ($P \leq 0.05$) in reducing the elevated levels of liver and kidney enzymes in compare to the others treating groups with red cabbage.

Previously red cabbage has been reported to have a wide scale of biological activities such as hepato-protective, anti-diabetic effects and hypocholesterolaemic due to it is rich in malvidin 5-glucoside and malvidin 3, 5-diglucoside which have a potent antioxidant effect which is explained by their radical scavenging properties (Wiczowski, *et al.*, 2013).

Komatsu, (1998) mentioned that red cabbage contains "S-Methyl-L-Cysteine Sulfoxide" which is able to prevent protein glycation, to inhibit activation of the polyol pathway, to cut off inflammatory processes and to ameliorate vascular complications in diabetes.

Table 5. Some liver and kidney function parameters of the experimental HFT/HFr rat groups

Groups Parameters	control		Treated with cabbage			
	-ve G1	+ve G2	2.5% G3	5% G4	250mg/kg G5	500mg/kg G6
AST(Iu/ml)	27.52 ±2.16 d	45.12 ±3.21 a	35.72 ±2.46 b	31.35±1.19 c	28.85 ±2.39 d	27.92±2.65 d
ALT(Iu/ml)	16.55 ±1.95 d	30.37 ±3.43 a	26.97 ±2.31 b	23.85 ±2.80 b	19.85 ±1.42 c	18.32±1.17 cd
Creatininmg/dl	0.85 ±0.02 d	2.74 ±0.21 a	2.11 ±0.08 b	1.94 ±0.16 c	1.01 ±0.09 d	1.00±0.09 d
Uric acidmg/dl	2.12 ±0.33 c	4.44 ±0.33 a	3.00 ±0.18 b	2.27 ±0.26 c	2.28 ±0.55 c	2.14±0.58 c
Ureamg/dl	24.80 ±1.26 d	48.10 ±3.08 a	34.25 ±2.29 b	27.85 ±1.19 c	25.47 ±1.38 d	25.37 ±1.32 d

Values with the same letters indicate insignificant difference and vice versa.

AST: Aspartate aminotransferase. ALT: Alanine aminotransferase.

Total antioxidant capacity (TAC), superoxide dismutase (SOD), nitric oxide (NO) were presented in Table (6). Results showed that high fat and high fructose diet reduced the activity of TAC and SOD in compare to (-v) group while it caused in elevation of nitric oxide level. On other hand, treating diabetic rats with red cabbage powder and extract had revealed those changes in compare to positive

control group. Our results were matched with (Jagdish, *et al.*, 2006), as he found that red cabbage reduce the inflammation inside animal rats which was caused by free radicals and it can preserve hepatic glutathione level. Also red cabbage could decrease the apoptotic β -cell rate by regulation of apoptotic signaling pathways in pancreas (Lee, *et al.*, 2015).

Table 6. Total antioxidant capacity (TAC), Superoxide dismutase (SOD) and nitric oxide (NO) of the experimental HFT/HFr rat groups

Groups Parameters	control		Treated with cabbage			
	-ve	+ve	2.5%	5%	250mg/kg	500mg/kg
(TAC) mmol/L	3.10±0.22 a	1.37±0.15 c	2.72±0.06 c	2.99±0.06 b	2.94±0.06 b	3.04±0.12 a
(SOD)U/MI	70.13±5.22 a	21.25±3.47 c	62.29±7.23 b	69.76±7.81 a	69.94±7.91 a	70.04±7.91 a
(NO) μ mol/l	2.68±0.33 c	9.45±1.44 a	4.02±1.05 c	3.76±1.21 b	3.46±1.21 c	3.17±1.21 c

Values with the same letters indicate insignificant difference and vice versa.

CONCLUSION

The search results explained to indicates that eating cabbage at different levels has a therapeutic protective effect against diabetes complications. The synergetic hypoglycemic effect is revealed by increased serum insulin levels, decreased serum glucose level as well as improvement of lipid profile. The results of the renal and liver function test have also established that the treated with cabbage read powder and extract at different levels lacks nephrotoxic and hepatotoxic effects by the high intake levels and duration of administration.

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تأثير أوراق الكرنب الأحمر كمخفض لسكر الدم ومضاد للأكسدة في الجرذان المصابة بالسكري التي تتغذى على نظام غذائي عالي الدهون/عالي الفركتوز

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تم استخدام ستة وثلاثون فأراً لدراسة تأثير مستخلص الكرنب الأحمر ضد مرض السكري الناتج عن نظام عالي الدهون/عالي الفركتوز. تم توزيع الفئران بشكل عشوائي إلى 6 مجموعات تحتوي كل منها (ن = 6). احتفظت المجموعة الأولى الطبيعية على نظام غذائي قياسي فقط. تمت تغذية المجموعات الست الأخرى على النظام الغذائي الأساسي ونظام عالي الدهون/عالي الفركتوز وأعيد تصنيفها إلى مجموعة تحكم إيجابية، الكرنب الأحمر تشمل مجموعات المسحوق والمستخلص (2.5، 5.0٪ من وزن النظام الغذائي الأساسي)، (200 مجم / كجم و 500 ملغم / كجم من وزن الجرذ). أظهرت نتائجنا زيادة معنوية في زيادة الوزن وتناول الطعام ونسبة كفاءة الغذاء في جميع المجموعات المعالجة بالكرنب الأحمر كمجموعات مسحوق ومستخلص مقارنة بمجموعة (+ve) (+ve)، وكذلك انخفاض معنوي في نسبة الجلوكوز في الدم ، HbA1c ، مؤشرات ملف الدهون ، المؤشرات الحيوية للكبد والكلية ومستويات أكسيد النيتريك (NO) ، زيادة كبيرة في الأنسولين ، HDL-c ، (TAC) ومستويات (SOD) في جميع المجموعات المعالجة بمسحوق الكرنب الأحمر عند مستوى 5٪ والمستخلص 500 مجم / كجم مقارنة بالمجموعة (+ve). تشير هذه الدراسة إلى أن العلاج بمسحوق الكرنب الأحمر ومستخلصه له تأثير مفيد مضاد للسكري وارتفاع دهون الدم .
الكلمات المفتاحية: الملفوف الأحمر ، ارتفاع دهون الدم ، السموم ، متلازمة الأيض.