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Biochemical and Nutritional Studies of Pulp, Seeds, and Peels of Citron Melon (C itrullus sp.) among Obese Albino Rats

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Abstract:

The objective of this study was to reduce obesity in rats fed a high-fat diet using citron melon plant parts. Fifty (50) male albino rats (Sprague- Dawley strain) (140±10g each) were used and divided into two main groups. The first main group (5 rats): negative control fed on a standard diet along with the experiment and the second main group (45 rats) (225 ± 20g), fed on a high-fat diet (10% cheap fat for 21 days to induce obesity) then were divided randomly into nine subgroups (5 rats each). Subgroup 1:positive control fed on a standard diet, subgroups 2,3, 4, 5, 6, 7, 8 & 9 were fed on a standard diet containing 2.5, 5% citron melon pulp (juice), 2.5, 5% citron melon seeds powder, 2.5, 5% citron melon peels powder, 2.5 and 5% mix of all parts (juice + powder), respectively. At the end of the experiment 28 days, the rats were sacrificed and blood samples were collected for analysis. In comparison with other experimental groups, the results indicated that diet 5% citron melon peels significantly decreased bodyweight, T.C, T.G, LDL, VLDL, AI, and blood glucose ($P \leq 0.05$), while diet 5% citron melon pulp revealed the highest increase in serum HDL level ($P \leq 0.05$) compared with control positive group. In conclusion, citron melon peels were effective in reducing body weight among obese rats, and they may be a promising supplement for bodyweight reduction.

Key words: *Watermelon, Cholesterol, Triglycerides, high-fat diet, body weight, lipid profile, glucose*

Introduction:

Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health (WHO, 2016). The global sharp rise in the prevalence of obesity has made it a critical public health issue in the last few decades. The obesity epidemic is the outcome of a multifaceted intricate interaction between environmental factors, genetic susceptibility and human behavior. It is certain that obesity is due to imbalance between energy intake and energy expenditure (Omer, 2020). Some drugs can result in rapid weight gain including antipsychotics, antidepressants, antihyperglycemics, antihypertensives and corticosteroids (Wharton et al., 2018). Diseases such as Cushing's disease and an underactive thyroid gland can lead to obesity. Polycystic ovary syndrome leading to insulin resistance may contribute to obesity (Koplemam et al., 2010). Long working hours can result in increased BMI due to reduced time for exercise and physical activity. It can also result in shifting towards ready processed meals and fast food rather than home-made meals (Kim et al., 2016). As rates of obesity rise so has interest in its associated complications and there is greater understanding of the role it plays in many diseases. This has led to fears that obesity-related complications such as diabetes, heart disease, dementia and cancer threaten to slow or even reverse the improvements in life expectancy seen over the past several decades (Wilding, 2012). Research on natural products and their anti-obesity activities has become a major topic of interest in the recent years (Schinzari et al., 2017).

The citron melon (Citrullus caffer) is a relative of the watermelon, also called Citrullus lanatus var. citroides (Nesom, 2011). It is in the family Cucurbitaceae which consists of various squashes, melons and gourds. The actual fruit of this plant resembles the more modern domesticated watermelons except that it is smaller and more spheroid. The meat of the melon is more whitish and dense much stronger in flavor (Laghetti and Hammer, 2007). Watermelon offers calories (6.4 g/100 g carbohydrates), water (93%), protein (high in seeds), fat (high in seeds), minerals (mainly phosphorus, iron, calcium and magnesium), vitamins (A, B, C and E) in good amounts (Maoto et al., 2019), soluble and insoluble fiber, fatty acids and amino acids. The chemical components of watermelon enhance its capacity to scavenge (LDL) and (HDL) in a cell membrane. A plethora of evidence shows that it can be effective for weight loss. (Aderiye et al., 2020). Watermelon (Citrulus lantus) has been used to treat various ailments such as cardio-vascular diseases, aging related ailments, obesity, diabetes, ulcers, and various types of cancers. The dietary intake of watermelon has proven benefits as functional food in humans for weight management Manivannan et al., (2020). Rind of watermelon not only contains plenty of health-promoting and blood-building chlorophyll, but actually contains important amino acid citrulline than the flesh. Citrulline is a non-protein amino acid and was first identified from watermelon. Citrulline is used in the nitric oxide system in humans and has antioxidant and vasodilatation roles (Rimando et al., 2005). Figueroa et al., (2011) revealed that the watermelon intake improved the arterial function and reduced the ankle blood pressure, brachial blood pressure and carotid wave reflection in obese middle-aged adults with pre-hypertension. Similarly, consumption of watermelon elicited the satiety response and reduced the body weight, body mass index (BMI) and waist to hip ratio in obese adults. Lum et al., (2019) suggested that watermelon can effectively reduce the appetite and aid in the weight management on comparison to the conventional refined carbohydrate snacks. Hong et al., (2018) decided that watermelon consumption has been linked to improve blood lipid profile in animals and humans.

The aim of this study is to investigate the efficiency of plant parts for decreasing weight loss, effect of plant parts on improving the lipid profile levels and clarify the effect of plant parts on serum blood glucose.

Materials and Methods:

Materials:

The plant: Citron melon (Citrullus Sp.) was collected from local region during August 2020.

The animals: Fifty (50) male albino rats (Spargue -Dawley strain) weighing (140 ± 10g) were obtained from Medical Insects Research Institute, Dokki, Giza, Egypt.

The standard diet: It was prepared from fine ingredients according to (Macia, 2006) and it was formulated to fulfill the dietary requirements given by (AIN, 1993).

The high fat diet (HFD): It was prepared from fine ingredients per 100g according to the following composition: fat 30% (tallow 15% + corn oil 15%), casein 12%, salts mixture 4%, vitamins mixture 1%, fiber 5%, methionine 0.3, choline chloride 0.2%, bile acid 0.2 and corn starch up to 100g according to (Moss, 1982).

Methods:

Preparation of pulp, seeds and peels of citron melon: Fresh fully-grown citron melon was washed with tap water and the outer skin (peel) removed (the thin green outer membrane of the plant) with a clean and sharp knife. The seeds were separated manually from the pulp. The pulp (the juicy part of the citron melon mostly white) was carefully squeeze in the electric mixer then kept in the freezer. The seeds were sieved to remove the bad and immature seeds and then separated from its coat. The seeds and the peels were sun-dried after which each was ground with a warring blender to fine powder and preserved in a dry clean container for preparing the experimental diet.

Rats: Rats were housed in individual wire cages in the animal house of Home Economics, Menoufia University under the normal laboratory condition and fed on basal diet for 4 consecutive days as adaptation period. Diets were introduced to rats in a special non-scattering feeding cup to avoid loss of food and contamination. Tap water was provided to rats by means of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage. Feed and water checked daily.

Induction of obesity in rats: Forty-five rats were fed on HFD for 21 days to achieve obesity. Experimental design: Fifty male albino rats were divided into the following groups: The first main group (n=5): Negative (-ve) control group, kept on a standard diet along the experiment and weighing (140 ± 10g). The second main group (n=45): Obesity induced group, rats were fed on (HFD) for 21 days to induce obesity and weighing (225 ± 20g). This main group was divided randomly into nine (9) subgroups (5 rats each) to fed on the experimental diets for (4) weeks according to the following: Subgroup 1: Positive (+ ve) control group (un treated group), subgroup (2): Obese rats, fed on a standard diet plus 2.5% Citron melon pulp (CMP) (juice), subgroup (3): Obese rats, fed on a standard diet plus 5% Citron melon pulp (CMP) (juice), subgroup (4): Obese rats, fed on a standard diet plus 2.5% Citron melon seeds (CMS) (powder), subgroup (5): Obese rats, fed on a standard diet plus 5% Citron melon seeds (CMS) (powder), subgroup (6): Obese rats, fed on a standard diet plus 2.5% Citron melon peels (CMPe) (powder), subgroup (7): Obese rats, fed on a standard diet plus 5% Citron melon peels (CMPe) (powder), subgroup (8): Obese rats, fed on a standard diet plus 2.5% mix of all parts and subgroup (9): Obese rats, fed on a standard diet plus 5% mix of all parts (juice + powder). Each of the above groups was kept in a single cage.

Blood samples: At the end of the experiment, blood samples were collected from the animals after fasted overnight at the end of the experiment using the abdominal aorta in which the rats were scarified under ether anesthetized. Blood samples were received into dry clean centrifuge tubes and left to clot at room temperature for half an hours then centrifuged for 10 minutes at 3000 r.p.m to separate the serum. Serum was carefully aspirated and transferred into clean quit fit plastic tubes and kept frozen at (-20) until the time of analysis and evaluation of some biochemical parameters (Malhotra, 2003).

Biological indices calculation: During the experimental period, the diet consumed was recorded every day and body weight recorded every week. The body weight gain (BWG %) and feed efficiency ratio (FER) were calculated according to Chapman et al., (1959) using the following equations:

Body Weight Gain (BWG) = Final weight (g) – Initial weight (g)×100/Initial weight (g).

Feed Efficiency Ratio (FER) = gain in body weight (g)/Feed intake (g).

Biochemical analysis of serum: Total cholesterol (T.C), triglycrides (T.G) and high density lipoprotein (HDL) were determined according to (Allain, 1974), Lopez (1977) and Fossati and Prencipe (1982) respectively. Determination of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were carried out according to the method of Lee and Nieman (1996) as follow:

LDL = Total Cholesterol – [(VLDL- C) + (HDL- C)].

VLDL = Triglycrides /5.

Atherogenic Index (AI) was calculated according to Nakabayashi et al., (1995) as follow:

Atherogenic Index (AI) = LDL+ VLDL / HDL.

Serum blood glucose was determined according to Young (2001).

Statically analysis: The data were statically using a computerized COSTAT program by one way ANOVA. The results are presented as mean ± SD. Difference between treatments at ($P \leq 0.05$) were considered significant (SAS, 1985).

Results and Discussion:

Data presented in table (1) show the total phenols in tested plant parts. It is evident that the highest total phenols content was in direct relation with citron melon peels followed by the seeds. It may be noticed that total phenols was widely variable among the used plant parts, the level in peels was 7 times more than that in pulp. This result seemed to be agree with Neglo et al., (2021) who reported that the peels contain the highest content of total phenolics followed by the seeds and the pulp has the lowest phenolic content so, the peels were found to possess the highest antioxidant activity whereas the pulp demonstrated the lowest.

Table (1): Total phenols of pulp, seeds and peels of citron melon

Sample name	Total phenols (mg/ml)
Citron melon pulp	59.84
Citron melon seeds	263
Citron melon peels	431.48

(FTRI, 2019).

Table (2) show the mean value of body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) of obese rats fed on various diets. The results indicated that positive control group had higher BWG and FER ($P \leq 0.05$) than negative control and obese groups treated with different parts of citron melon. These results agreed with the results obtained by Mohammed, (2016) and Hosny, (2017) who found that BWG and FER of rats feeding on diet contained high fat were increased when compared to control (-) group. Feeding obese rats on different parts of citron melon led to decrease BWG and FER compared with positive control. Also, obese rats fed on 2.5% citron melon pulp and 5% citron melon peels had the same effect on FI. These results had the same trend with Ware et al., (2015) who indicated that eating watermelon can decrease the risk of obesity and lower weight. Also, Aderiye et al., (2020) showed that watermelon can be effective for weight loss. Moreover, Manivannan et al., (2020) reported that the dietary intake of watermelon has been used to treat obesity and weight management. In the same table, the highest FI was observed in obese rats treated with 5% citron melon pulp.

Table (3) show the effect of citron melon parts on lipids profile of obese rats fed on various diets. The results indicated that obesity due to HFD caused increase T.C, T.G, LDL, VLDL, and AI but decreased the serum HDL. Rats fed on (CMPe) as 5% showed the best treatment for lowering T.C, T.G, LDL, VLDL and AI. The highest increase in serum HDL level was revealed for obese rats fed on 5% (CMP) and recorded the best treatments.

Table (2): Effect of Citron melon parts on BWG, FI and FER of obese rats

Variable	Control – ve	Obese groups									LSD P ≤ 0.05
		Control + ve	CMP (2.5%)	CMP (5%)	CMS (2.5%)	CMS (5%)	CMPe (2.5%)	CMPe (5%)	Mix (2.5%)	Mix (5%)	
BWG (g/da)	1.14[b] ± 0.003	1.68[a] ± 0.020	-0.72[g] ± 0.010	-1.04[h] ±0.005	0.54[d] ± 0.020	0.89[c] ± 0.010	-1.30[i] ± 0.040	-1.54[j] ±0.003	-0.213[e] ± 0.001	-0.563[f] ± 0.002	0.028
FI (g/da)	17.00[g] ± 0.200	18.48[c] ± 0.003	17.92[d] ± 0.10	19.90[a] ± 0.040	16.39[i] ± 0.020	17.49[e] ±0.005	17.25[f] ±0.006	17.96[d] ± 0.002	17.69[h] ± 0.010	19.02[b] ± 0.020	0.111
FER (g/da)	0.067[b] ± 0.011	0.091[a] ± 0.008	-0.040[f] ± 0.003	-0.052[g] ±0.002	0.033[d] ±0.012	0.051[c] ± 0.007	-0.075[h] ± 0.001	- 0.086[i] ± 0.002	- 0.012[e] ± 0.001	-0.030[f] ± 0.004	0.011

Means in the same row with different letters are significantly different and vice versa at (p≤0.05).

The data of table (3) are in the same line with obtained by Massa et al., (2016) reported that daily supplementation of watermelon extract for 42 days reduced plasma T.C and LDL. Zafar et al., (2016) decided that watermelon Citrullus lanata has anti-hyperlipidemics activity. Biswas et al., (2017) stated that watermelon seeds decreased serum T.G, T.C, VLDL, LDL and AI and increased HDL in hyperglycemic and hyperlipidemic rats. Abu-Hiamed (2018) reported that feeding hypercholesterolemic rats with 10% watermelon rind led to significant reduction in serum levels of T.C and LDL. Aminu et al., (2018) found that daily intake of extract from a blend of seed and pulp of Citrullus lanatus fruits for 14 days can reduce T.G and LDL levels in normal albino rats. Hong et al., (2018) showed that watermelon and l-arginine supplementation improved lipid profiles in rats fed an atherogenic diet by lowering serum concentrations of T.G, T.C and LDL.

Table (3): Effect of Citron melon parts on serum T.C, T.G, HDL, LDL, VLDL and AI in obese rats

Variable	Control – ve	Obese groups									LSD P ≤ 0.05
		Control + ve	CMP (2.5%)	CMP (5%)	CMS (2.5%)	CMS (5%)	CMPe (2.5%)	CMPe(5%)	Mix (2.5%)	Mix (5%)	
(T.C) (mg/dl)	102.80[i] ± 0.01	237.80[a] ± 0.12	153.80[d] ± 0.03	131.00[g] ± 0.01	178.30[c] ± 0.05	190.30[b] ± 0.11	103.80[h] ± 0.04	96.30[j] ±0.2	140.80[e] ± 0.07	137.80[f] ± 0.03	0.105
(T.G) (mg/dl)	41.80[j] ± 0.011	148.00[a] ± 0.090	89.80[e] ± 0.060	71.20[g] ± 0.070	110.10[c] ± 0.040	117.50[b] ± 0.110	70.20[h] ± 0.030	56.50[i]± 0.034	88.60[f] ± 0.020	91.20[d] ± 0.110	0.114
(HDL-C) (mg/dl)	52.60[a] ± 0.045	37.00[i] ± 0.030	42.40[e] ± 0.011	49.60[b] ± 0.091	38.50[h] ± 0.113	41.50[f] ± 0.010	45.80[c] ± 0.121	42.80[d] ±0.009	40.80[g] ± 0.025	42.50[e] ± 0.020	0.108
(LDL-C) (mg/dl)	41.84[j] ± 0.003	171.20[a] ± 0.020	93.44[d] ± 0.003	67.16[g] ± 0.005	117.78[c] ± 0.011	125.30[b] ± 0.090	43.96[h] ± 0.070	42.20[i] ±0.021	82.23[e] ± 0.004	77.06[f] ±0.054	0.070
(VLDL-C) (mg/dl)	8.36[j] ± 0.009	29.60[a] ± 0.110	17.96[e] ± 0.016	14.24[g] ± 0.007	22.02[c] ± 0.019	23.50[b] ± 0.021	14.04[h] ± 0.014	11.30[i] ±0.021	17.72[f] ± 0.013	18.24[d] ±0.018	0.065
(AI) (mg/dl)	0.95[h] ± 0.017	5.43[a] ± 0.093	2.63[c] ± 0.011	1.64[f] ± 0.009	3.63[b] ± 0.012	3.59[b] ± 0.013	1.04[g] ± 0.018	0.53[i] ±0.012	2.45[d] ± 0.018	2.24[e] ±0.011	0.055

Means in the same row with different letters are significantly different and vice versa at (p≤0.05).

Table (4) show the mean value of glucose of obese rats fed on various diets. The mean value of control (+) group was higher than control (-) group. The lowest increase in glucose level was revealed for obese rats fed on 5% (CMPe) and recorded the best treatments.

The results of table (4) are confirmed by many authors. Alexandra (2018) indicated that daily intake of all watermelon products for 10 weeks improved fasting blood glucose and circulating serum insulin concentrations for mice fed a high-fat, high-cholesterol and high-sucrose diet modeling a western-style diet. Okafor and Elemuo (2018) decided that the ethanoic extract of watermelon seed has hypoglycaemic properties in alloxan induced diabetic rats and can be employed in the treatment of diabetics. Ahangarpour et al., (2020) showed that using of Citrullus colocynthis plant on type II diabetic patients through dermal absorption could significantly decrease blood glucose levels, stimulate insulin secretion and improve the function of pancreatic beta cells. Ajiboye et al., (2020) reported that watermelon (Citrullus lanatus) juice has been shown to possess antidiabetic activities as it reduces the fasting blood glucose level, serum lipid profile, glucose-6-phosphatase, lipid peroxidation and anti-inflammatory activities in alloxan-induced diabetic rats. Becraft et al., (2020) showed that intake of whole watermelon products improved fasting blood glucose and circulating serum insulin concentrations in obese male mice whereas fiber-rich additives made from watermelon rind and skin improved glucose metabolism and energy efficiency. Manivannan et al., (2020) stated that watermelon (Citrulus lantus) has been used to treat diabetes.

Table (4): Effect of Citron melon parts on serum blood glucose (mg/dl) in obese rats

Variable	Control – ve	Obese groups									LSD P ≤ 0.05
		Control + ve	CMS (2.5%)	CMS (5%)	CMS (2.5%)	CMS (5%)	CMPe (2.5%)	CMPe (5%)	Mix (2.5%)	Mix (5%)	
Glucose (mg/dL)	47.80[j] ± 0.020	92.00[a] ± 0.060	62.00[f] ±0.040	60.20[g] ±0.030	70.50[b] ±0.010	68.60[c] ±0.035	54.60[h] ±0.020	53.80[i] ±0.010	64.00[d] ±0.040	63.40[e] ±0.110	**0.080**

Means in the same row with different letters are significantly different and vice versa at (p≤0.05).

Conclusion

Watermelons are very good source of important nutritive components for human consumption. It also contains different components of medicinal values and it would be more effective in healthcare management. For the treatment of obesity and overweight and to improve the serum lipids profile and glucose, it is recommended to use the plant peels.

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دراسات بيوكيماوية وتغذوية على لب، بذور وقشور بطيخ الكاوتش باستخدام الفئران البدينة.

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قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي، جامعة المنوفية، شبين الكوم، مصر

الملخص العربي:
الهدف من هذه الدراسة هو تقليل السمنة فى الفئران التي تغذت على نظام غذائي عالي الدهون باستخدام أجزاء نبات بطيخ الكاوتش. تم إستخدام خمسين (٥٠) من ذكور الفئران البيضاء (سلالة Sprague- Dawley) (١٤٠±١٠ جم) وتم تقسيمهم إلى مجموعتين رئيسيتين. المجموعة الرئيسية الأولى (٥ فئران) : ضابطة سالبة وتغذت على الغذاء القياسى طوال التجربة والمجموعة الرئيسية الثانية (٤٥ فأر) (٢٢٥±٢٠ جم): تغذت على غذاء عالى الدهون (١٠% دهون حيوانية لمدة ٢١ يوم لإحداث السمنة) ثم قسمت عشوائيا الى ٩ مجموعات فرعية (٥ فئران للمجموعة). المجموعة الفرعية ١: ضابطة موجبة وتغذت على الغذاء القياسى. تم تغذية المجموعات الفرعية ٢،٣، ٤، ٥، ٦، ٧، ٨ و ٩ على وجبة قياسية تحتوى على ٢.٥ ، ٥% لب البطيخ (عصير) ، ٢.٥، ٥% بذورالبطيخ (مسحوق) ، ٢.٥، ٥% قشورالبطيخ (مسحوق) ، ٢.٥ ، ٥% خليط من جميع الأجزاء (عصير+مسحوق) ، على التوالى. فى نهاية التجربة 28 يوم تم ذبح الفئران وجمع عينات الدم للتحليل. أشارت النتائج إلى أن النظام الغذائى المدعم بنسبة ٥% من قشور بطيخ الكاوتش أدى الى انخفاض معنوى فى وزن الجسم ، AI, VLDL, LDL, T.G , T.C و جلوكوز الدم ($P \leq 0.05$) بينما أظهر النظام الغذائي المدعم بنسبة 5٪ من عصير لب بطيخ الكاوتش أعلى زيادة في مستوى HDL في الدم ($P \leq 0.05$) مقارنة بالمجموعة الضابطة الموجبة. تظهر هذه الدراسة أن قشور بطيخ الكاوتش يمكن أن تكون فعالة في إنقاص الوزن مما يحسن بعض العوامل المرتبطة بزيادة الوزن والسمنة.

الكلمات المفتاحية: بطيخ الكاوتش، نظام غذائي عالي الدهون، وزن الجسم، الدهون، جلوكوز الدم.