

Effect of Grilled Meat Supplemented with Cinnamon, Green Coffee and Cardamom on Body Weight of Obese Rats.

***Naeem M. Rabeh . Hany G. EL-Masry.Eman M.Ragheb
and Shimaa F. Mahmoud***

Nutrition and Food Science Department, Faculty of Home Economics
Helwan University, Cairo, Egypt

Abstract

Cinnamon, green coffee and Cardamom are famous for having health-promoting properties due to active phytochemicals contents, The aim of the present study was to investigate the effect of grilled meat supplemented with cinnamon, green coffee, and cardamom on body weight status of obese rats. Forty-two adult male rats were divided into two main groups; the first main group (6 rats) fed on basal diet (as a control negative group), The second main group (36 rats) fed on high fat diet for four weeks then divided as follows: Subgroup (1) fed on high fat diet (as a control positive group). Subgroup (2) fed on high fat diet containing half amount of protein from grilled meat without any supplementation. Subgroups (3 to 5) fed on high fat diet containing half amount of protein from grilled meat supplemented with either cinnamon, green coffee, or cardamom at the level of 5% respectively. Subgroups (6) fed on high fat diet containing half amount of protein from grilled meat supplemented with 5% of mixture, cinnamon, coffee, and cardamom (one third amount of each herb). At the end of the experimental period (8 weeks), animals were sacrificed for blood collection. The results indicated that groups fed on high protein diet (grilled meat) with either cinnamon, green coffee, cardamom, or their mixture had significant decrease ($P < 0.05$) in their final body weight, Leptin hormone as well as the mean levels of lipid profile as well as high density lipoprotein was significantly increased ($P < 0.05$) compared to the positive control group. The study recommends that intake of grilled meat with either cinnamon, green coffee, cardamom, or their mixture could be beneficial on trial for patients who suffer from obesity.

Benefit: Cinnamon, green coffee, Cardamom, lipid profile, obesity, rats

Introduction

Obesity is now a worldwide epidemic, with an estimated 57.8% of adults worldwide expected to be classified as obese by 2030 according to figures released by the World Health Organization. Obesity is characterized by an excessive accumulation of body fat that gives rise to significant comorbidities, such as diabetes, hypertension, dyslipidemia, cardiovascular disease, and many cancers (*Ortega and Lavie, 2018*).

Obesity is considered a principal public health concern and ranked as the fifth foremost reason for death globally. Overweight and obesity are one of the main lifestyle illnesses that leads to further health concerns and contributes to numerous chronic diseases, including cancers, diabetes, metabolic syndrome, and cardiovascular diseases. The World Health Organization also predicted that

30% of deaths in the world will be initiated with lifestyle diseases in 2030 and can be stopped through the suitable identification and addressing of associated risk factors and behavioral involvement policies. Thus, detecting and diagnosing obesity as early as possible is crucial (**Mahmood et al.,2021**). Dietary protein is effective for body-weight management (**Neda et al., 2020**), in that it promotes satiety, energy expenditure, and changes body-composition in favor of fat-free body mass. High-protein diets are more satiating than diets lower in protein. Furthermore, subjects consumed less food during an *ad libitum* high-protein diet relative to baseline, while being similarly satiated and satisfied (**Mathijs et al.,2018**).

Cinnamaldehyde, cinnamic acid, and cinnamate, found in Cinnamon play an essential role in its natural antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, and cholesterol-lipid-lowering properties (**Fahadah et al.,2020**).

Coffee is among the most-consumed beverages in the world. Caffeine and other bioactive compounds, as polyphenol and chlorogenic acid that found in coffee have been suggested to confer diverse health benefits (**Ariel et al., 2019**). It was found that as the individuals; coffee consumption increased (from 1.5 cups to 3 cups per day or more), the risk of metabolic syndrome associated with particularly lower triglyceride levels decreased (**Takami et al., 2013**).

The major use of cardamom is culinary purpose for flavoring food. It is also used in medicine as an aromatic stimulant, carminative and flavoring agent (**Kishorbhai et al., 2018**). Cardamom capsules are combined with cinnamon and long pepper to treat obesity, glycemic imbalance, liver, kidney and heart diseases (**Kaliyaperumal et al., 2020**).

This study was conducted to evaluate the effect of grilled meat supplemented with cinnamon, green coffee, cardamom and their mixture on body weight of obese rats.

Materials and Methods

Materials:

Casein, vitamins, minerals and cellulose were purchased from El-Gomhoria company – Cairo – Egypt. Cinnamon, green coffee and cardamom were obtained from the local market. Beef tallow and beef meat were obtained from the local market. Adult male albino rats (Sprague- Dawley strain) (n=42 rat) weighing approximately (150 ± 5g) were purchased from Helwan Experimental Animals Station. Kits for blood analysis were purchased from Gama Trade Company for Chemical, Cairo, Egypt. The study was carried out at the Animal House of Faculty Home Economics, Helwan University.

Methods:

Preparation of grilled beef meat: Beef was purchased from the local market. Meat was sliced 2 cm thick. The steaks divided into 5 groups: The first group was cooked by grilling without any additions. Groups(2 to 5): the beef meat was mixed with powdered cinnamon (5%), green coffee (5%), cardamom (5%) and mixture of cinnamon, coffee, and cardamom (one third amount of each herb) with 5%, respectively, put in a refrigerator for 12 hours, then cooked by grilling. These grilled steaks with or without supplementation were dried at 50 °C.

Induction of obesity: Rats were fed four weeks on high fat diet (containing: casein 14%, cellulose 5%, vitamin mixture 1%, minerals mixture 3.5%, sucrose 10%, (beef tallow 19% + corn oil 1%), l-cystine 0.18, choline bitartrate 0.25% and the remainder was starch to induce obesity in rats (*Min et al., 2004*).

Biological study : Forty-two adult male rats housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. All diets formulated to cover the nutrient requirements of the rats following the recommendations of the American Institute of Nutrition (AIN-93M) (*Reeves et al., 1993*). After this week rats were divided in to two main groups as follows:

The first main group (6 rats) was fed on basal diet (as a control negative group).

The second main group (36 rat) was fed on high fat diet for four weeks then divided as follows:**Subgroup (1)** was fed on high fat diet (as a control positive group). **Subgroup (2)** was fed on high fat diet containing half amount of the protein from grilled meat without any supplementation. **Subgroups (3 to 6)** were fed on high fat diet containing half amount of the protein from grilled meat supplemented with 5% of either cinnamon, green coffee, cardamom or mixture of cinnamon, coffee and cardamom (one third amount of each herb) with 5%, respectively.

Feed intake was recorded daily and animals were weighed at the beginning and twice a week throughout the experimental period. Body weight gain (BWG) % and feed efficiency ratio (FER) were calculated at the end of the experiment (8 weeks), according to the method of (*Chapman et al., 1959*).

Biochemical analysis: Rats were fasted over night before scarifying and the blood samples were collected from each rat then were centrifuged to obtain serum .Serum was analyzed to determine the following parameters :Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein-cholesterol (HDL-C) were determined according to (*Allain, 1974; Fassati and prencipe, 1982 and Lopez, 1977*), respectively. Low density lipoprotein –cholesterol (LDL-C) and very low density lipoprotein (VLDL-C) were determined according to the equation of (*Friedwable et al., 1972*).
 $VLDL-C = TG/5$, $LDL-C = TC - (HDL-C + VLDL-C)$. Leptin hormone was determined using enzyme-linked immune - sorbent (ELISA) assay (*Xiong et al., 2005*).

Statistical Analysis: All obtained data was analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20. Analysis of Variance \pm (ANOVA) test was used for determining the significances among different groups. All differences were considered significant at $P < 0.05$ (*Armitage and Berry, 1987*).

Results and Discussions

The mean value of initial body weight (IBW) in the obese rats was increased significantly ($p < 0.05$), as compared to the mean value of IBW in healthy rats (control –ve group). The final body weight was significantly increased ($P < 0.05$) in the (control+ve) as compared to the control –ve group. However, all treated groups had a significant ($P < 0.05$) decrease in final body weight (FBW) as compared to (control+ve). Moreover, there are a significant difference in FBW among the treated groups. The most effective FBW reduction was recorded at the group fed on the mixture of tested materials.

From table (1) it could be observed that, significant rise in BWG% and FER in the positive control group fed on high fat diet compared with the negative control group fed on basal diet.

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The mean value of BWG% and FER of all treated groups which were fed on high protein diet with 5% of either dried cinnamon, green coffee, cardamom or 5% of the mixture cinnamon, green coffee, cardamom (one third amount of each herb) were decreased significantly ($p < 0.05$), compared with the positive control group. There was no significant difference in BWG% or FER between the groups fed on grilled meat with either cinnamon or cardamom. In addition, the BWG% and FER were decreased significantly compared with the group treated with grilled meat without any supplementation.

The highest reduction in BWG% or FER was observed at the group fed on a grilled meat with a mixture of the tested materials followed by green coffee group. Feed intake revealed a decrease among the treated groups with the tested materials when compared to the +ve control.

Our findings were in agreement with a recent systematic review which proposed the potential anti-obesity effects of cinnamon supplementation meta-analysis of randomized controlled trials (RCTs), flavanols, a major component of cinnamon, showed a potential role against obesity (**Mollazadeh and Hosseinzadeh, 2016**). Such results were also observed with polyphenols (**Farhat et al., 2017**). In addition to its effect on body weight, cinnamon supplementation have improved weight-related disorders and including blood triglycerides, total cholesterol, HDL-C levels, fasting plasma glucose, and Glycated hemoglobin (HbA1c) levels in humans (**Maieran et al., 2017**). The beneficial effects of cinnamon supplementation on inhibiting pancreatic amylase and reducing intestinal glucose absorption, stimulating cellular glucose uptake and glycogen synthesis, inhibiting gluconeogenesis, stimulating insulin receptor activity, and improving weight loss were also seen in vitro and in vivo studies (**Ranasingheet al., 2013**). Flavonoids and phenolic complexes like epicatechin, catechin, and procyanidin B2 in cinnamon can decrease the absorption of glucose in the intestine, decrease glycogenolysis, increase glycogen synthesis, and decrease chylomicron absorption that leads to reduction in the synthesis and storage of fat and improvement in anthropometric measures (**Seyed et al., 2020**).

The above-mentioned results agreed with the finding of **Chiao-Nan et al., (2021)** who showed that combined high-protein diet and exercise intervention significantly decreased fat mass and improved lipid profiles, insulin sensitivity, in middle-aged adults with obesity. Also, the results are in the line with (**Joohee and Hyun, 2022**) who showed that rats fed on normal or high fat diet with 1% cinnamon extracts had lower final body weight and body weight gain. Moreover, **Seyed et al., (2020)** indicated that cinnamon supplementation at the dosages of 2 g/d, when administered for 12 weeks can significantly reduce body weight, body Mass Index, and fat mass, hence they, recommended that cinnamon as a supplement has weight-reducing effect in obesity management (**Seyed et al., 2020**).

Mehdi et al., (2019) reported beneficial effects of green coffee extract on weight and blood glucose management and metabolism of lipids. Green coffee extract reduces the fat reserves in adipocytes. Moreover, **Cimi et al., (2020)** reported that low dose green coffee extract has a beneficial effect on body weight.

Green coffee beans are valued as functional ingredients with several health benefits including weight loss (**Roshan et al., 2018**). These results agreed with the obtained results in this study **Sahar et al., (2020)** showed that the administration of green cardamom is a beneficial approach for improving of anthropometric, glycemic and androgen hormones, as well as, obesity in Polycystic ovary

syndrome (PCOS) in women.

Moreover after 2 months intervention with three gram cardamom, weight, BMI, WC, insulin sensitivity were significantly decreased compared to control group (*Fatemeh et al., 2017*). Another trial showed that green cardamom is rich in flavonoids and isoflavones which are contributed in reducing insulin resistance by decreasing adipose tissue storage(*Aghasi et al.,2019*).

**Table (1):
The effect of high protein diet(grilled meat) with cinnamon, green coffee, cardamom and their mixture on body weight status of obese rats:-**

Groups	Parameters	IBW(g)	FBW(g)	BWG%	FI (g/d/rat)	FER
Control(-ve)		170.93±2.41 ^b	186.76±0.62 ^b	16.10±1.88 ^b	14.00	0.041±0.04 ^a
Control(+ve)		208.86±1.27 ^a	254.13±1.57 ^a	21.67±0.26 ^a	20.00	0.050±0.01 ^a
Grilledmeat		205.36±3.44 ^a	176.50±1.32 ^c	-14.02±0.80 ^c	15.00	-0.043±0.03 ^b
Grilledmeatwith 5% cinnamon		202.23±2.38 ^a	160.93±2.69 ^e	-20.43±0.52 ^d	13.50	-0.068±0.01 ^c
Grilledmeat With 5% greencoffee		203.67±3.17 ^a	152.67±2.02 ^f	-28.63±1.40 ^e	13.00	-0.100±0.05 ^d
Grilled meat with5% cardamom		206.43±2.63 ^a	166.66±2.02 ^d	-19.25±0.64 ^d	13.70	-0.065±0.02 ^c
Grilled meat and herbs mixture		204.66±2.02 ^a	137.36±1.33 ^g	-32.76±0.70 ^f	12.20	-0.122±0.03 ^e

All results are expressed as mean± SE.

Values in each column which have different letters are significantly different (p<0.05).

The effect of high protein diet (grilled meat) with cinnamon,green coffee, cardamom and their mixture on lipid profile on obese rats:-

Data in **Table(2)** showed the effect of high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture on lipid profile in obese rats . Results revealed that serum TC, TG, LDL-C and VLDL-C were increased significantly while HDL-C was significantly decreased (P< 0.05) in the positive control group compared with the negative control group. On the other hand, the groups fed on high protein diet (grilled meat) with either cinnamon, green coffee, cardamom or their mixture had more lower lipid profile than the group fed on high protein diet (grilled meat) only.All treated groups had significant decrease (p<0.05) in the mean value of lipid profile as compared to +ve control group.

The highest decrease in lipid profile and highest increase in HDL-C was observed in the group fed on high protein diet with the mixture of dried (cinnamon, green coffee, cardamom) then the group fed on high protein diet (grilled meat) with cinnamon.The results are in harmony with (*Navid et al.,2020*) who reported that cinnamon supplementation significantly decreased serum TG, TC, and LDL-C concentrations, but did not affect HDL-C levels, in patients with type 2 diabetes. *Lee et al., (2003)* reported that the main hypolipidemic effect of cinnamon is exerted by inhibition of hepatic HMG Co-A reductase enzyme. Further, cinnamon possesses lipolytic activity, via decreasing insulin

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resistance and consequent improvement of intestinal apoB48-containing lipoproteins overproduction. In addition, cinnamon contains high levels of polyphenolic compounds that have hypocholesterolemic activity via the intestinal absorption inhibition of cholesterol.

Green coffee extract supplementation improved TC. Also, there was a significant improvement in other markers of lipid profile in some subgroups of clinical trials (*Aliyar and Omid, 2020*). Also, *Cimi et al., (2020)* confirmed that low dose green coffee extract lowers total serum cholesterol, triglyceride, LDL, and TNF- α levels in high-fat diet induced obese rats. *Omid et al., (2020)* reported that green coffee extract (GCE) supplementation led to a significant reduction in serum TC, particularly in individuals with elevated levels of TC.

In addition cardamom supplementation might be effective in decreasing blood TG (*Parivash et al., 2019*). Another mechanism of cardamom effects on serum lipids may be through its effects on peroxisome proliferator-activated receptor- α (PPAR α). PPAR α activation has been proposed to up regulate genes involved in lipid oxidation in skeletal muscle, hepatic lipids, and lipoprotein metabolism (*Harrington et al., 2007*). The results in this study agreed with the previous studies.

Table (2):
The effect of high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture on lipid profile on obese rats:-

Parameters Groups	TC	TG	VLDL-C	HDL-C	LDL-C
	(mg/dl)				
Control(-ve)	99.52 \pm 1.89 ^g	75.16 \pm 1.78 ^f	15.03 \pm 0.35 ^f	59.96 \pm 1.49 ^a	24.52 \pm 0.86 ^g
Control(+ve)	151.63 \pm 1.29 ^a	129.26 \pm 1.23 ^a	25.85 \pm 0.24 ^a	27.93 \pm 0.85 ^e	97.84 \pm 1.91 ^a
Grilled meat	142.91 \pm 1.06 ^b	123.100 \pm 1.10 ^b	24.62 \pm 0.22 ^b	34.00 \pm 1.52 ^d	84.29 \pm 0.28 ^b
Grilled meat with 5% cinnamon	119.10 \pm 0.85 ^e	107.87 \pm 1.18 ^d	21.57 \pm 0.23 ^d	43.36 \pm 1.21 ^c	54.15 \pm 1.43 ^e
Grilled meat with 5% green coffee	129.33 \pm 1.73 ^d	112.00 \pm 0.81 ^c	22.40 \pm 0.16 ^c	38.01 \pm 1.39 ^d	68.92 \pm 1.18 ^d
Grilled meat with 5% cardamom	134.83 \pm 1.12 ^c	119.50 \pm 1.70 ^b	23.90 \pm 0.34 ^b	34.37 \pm 0.89 ^d	76.56 \pm 0.54 ^c
Grilled meat and mixture	112.52 \pm 1.14 ^f	95.06 \pm 1.57 ^e	19.01 \pm 0.31 ^e	54.57 \pm 2.00 ^b	38.94 \pm 2.71 ^f

All results are expressed as mean \pm SE.

Values in each column which have different letters are significantly different ($p < 0.05$).

Serum leptin: Leptin levels in the positive control group was significantly increased ($P < 0.05$) compared with the normal group (table 3). On the other hand, all treated groups with high protein diet (grilled meat) only and groups fed on high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture had significant ($P < 0.05$) decrease in leptin hormone compared with the positive control group. There was a significant difference in leptin hormone among all the treated

groups. The highest decrease in leptin hormone was observed in the group fed on high protein diet with the mixture of (cinnamon, green coffee, cardamom) followed by the group fed on high protein diet (grilled meat) with cinnamon. Moreover, leptin hormone decreased significantly at the treated groups with the supplemented materials as compared to the group fed on grilled meat only.

Andrea et al.,(2019) reported that Leptin hormone is capable of reducing food intake and body weight, and was initially considered for use in the treatment of obesity. However, obese subjects have high levels of circulating leptin and to be insensitive to the exogenous administration of leptin. This results agreed with the study of the current results. In addition the combination of an oat β glucan extract with decaffeinated phenolic green coffee extract appears to increase satiety and reduce appetite, which is related with higher levels of leptin (acute effect) and lower ghrelin levels (sustained effect), respectively, in addition to lower hunger sensation (**Mónica et al., 2021**).

Concerning leptin hormone, the present results revealed that rats fed on high fat-diet (HFD) had high serum leptin hormone level when compared with those fed on basal diet (**Neveen, 2014**). This finding agreed with that reported by **Huang et al.,(2004)**, who found that HFD increased serum leptin level in rats. Leptin plays a key role in regulating energy intake and energy expenditure and the level of circulating leptin is proportional to the total amount of body fats. Cinnamon and ginger extracts significantly decreased serum leptin levels in obese diabetic rats. This result agreed with that of **Shatwan et al.,(2013)**, who reported that cinnamon extract reduced body weight, decreased serum leptin level and depressed appetite in obese rats fed on HFD. The authors concluded that cinnamon may be useful in the treatment of obesity and related disorders as anti-obesity agent. Increasing adipose tissue causes the production of the hormone leptin. Leptin is a hormone encoded by the obesity gene (LPER) on human chromosome 7 (**Chow et al.,2017**). High levels of this hormone are seen in women, which prevent the conversion of androgen to estrogen and subsequent follicular atresia (**Zeng et al., 2020**). Therefore, it seems that the green cardamom with anti-inflammatory properties and reduced fat storage has beneficial effects in improving the status of androgen hormones (**Sahar et al.,2021**).

Conclusion

Grilled meat with cinnamon, green coffee, cardamom and their mixture could be beneficial on trial for patients who suffer from obesity.

Table (3):
Effect of high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture on serum leptin of obese rats.

Groups	Parameters	Leptinhormone (Ug/l)
Control(-ve)		8.39±0.49 ^g
Control (+ve)		33.20±0.93 ^a
Grilled meat		29.86±0.77 ^b
Grilled meat with 5% cinnamon		19.37±0.65 ^e
Grilled meat with 5% greencoffee		22.72±0.99 ^d
Grilled meat with 5% cardamom		26.24±0.74 ^c
Grilled meat and mixture		15.43±0.89 ^f

All results are expressed as mean ± SE.

Values in each column which have different letters are significantly different ($p < 0.05$).

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تأثير اللحوم المشوية و المدعمة بالقرفة والقهوة الخضراء والهيل على وزن الجسم
فى الفئران المصابة بالسمنة

نعيم محمد رابع ، هانى جابر المصرى ، ايمان محمود راجب
شيماء فاروق محمود

قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي - جامعة حلوان

الملخص العربى

تشتهر القرفة والقهوة الخضراء والهيل بخصائصها المعززة للصحة بسبب محتوياتها من المواد الكيميائية النباتية النشطة. لذلك كان الهدف من هذه الدراسة هو معرفة تأثير اللحوم المشوية المدعمة بالقرفة والقهوة الخضراء والهيل على وزن الفئران المصابة بالسمنة. تم تقسيم اثنين وأربعين ذكور الفئران البالغة إلى مجموعتين رئيسيتين المجموعة الأولى (6 فئران) تتغذى على النظام الغذائي الأساسي (كمجموعة ضابطة سلبية). المجموعة الرئيسية الثانية (36 فأراً) تغذت على نظام غذائي عالي الدهون لمدة أربعة أسابيع ثم قسمت على النحو التالي ، المجموعة الفرعية (1) تغذت على نظام غذائي عالي الدهون (كمجموعة تحكم إيجابية). المجموعات الفرعية (2) تتغذى على نظام غذائي عالي الدهون يحتوي على نصف كمية البروتين من اللحوم المشوية دون أي مكملات. المجموعات الفرعية (من 3 إلى 5) تتغذى على نظام غذائي غني بالدهون يحتوي على نصف كمية البروتين من اللحم المشوي المضاف إليه القرفة والقهوة الخضراء والهيل بنسبة 5% على التوالي. تتغذى المجموعات الفرعية (6) على نظام غذائي غني بالدهون يحتوي على نصف كمية البروتين من اللحوم المشوية مدعمة بنسبة 5% من الخليط (القرفة والقهوة والهيل). في نهاية فترة التجربة (8 أسابيع) تم تشريح الفئران و الحصول على السيرم . أشارت النتائج إلى انخفاض معنوي في الوزن النهائي للمجموعات التي تغذت على نظام غذائي عالي البروتين (اللحوم المشوية) في وزن الجسم النهائي وهرمون (p<0.05) المشوية) سواء بالقرفة أو البن الأخضر أو الهيل أو الخليط (p<0.05) وهرمون اللبتين وكذلك في متوسط مستويات الدهون بينما زاد البروتين الدهني عالي الكثافة معنوياً مقارنة بمجموعة التحكم الموجبة

وتوصي الدراسة بأن تناول نظام غذائي عالي البروتين (اللحوم المشوية) مع القرفة أو القهوة الخضراء أو الهيل أو خليطها قد يكون مفيداً للمرضى الذين يعانون من السمنة لذا يفضل تجربته على الانسان.

الكلمات المفتاحية: القرفة ، البن الأخضر ، الهيل ، الدهون ، السمنة ، الفئران.