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Effect of grilled meat supplemented with cinnamon, green coffee and cardamom on serum liver function of obese rats

Naeem M. Rabeh

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

Hany G. EL-Masry

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

Eman M. Ragheb

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

Shimaa F. Mahmoud

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

Abstract---Obesity has become a global epidemic, and it is a major risk factor for other metabolic disorders such as type 2 diabetes and cardiometabolic disease. Dietary protein is effective for body-weight management. This study aimed to investigate the effect of grilled meat supplemented with cinnamon, green coffee and cardamom on serum liver function of obese rats. Forty-two adult male rats weighing approximately (150 ± 5 g.) fed on basal diet for one week for adaptation. After this week rats 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 rat) fed on high fat diet for four weeks then divided as follows: Subgroup (1) fed on high fat diet (as a control positive group). Subgroups (2) fed on high fat diet containing half amount of protein from grilled meat without any supplementation. Subgroups (3) fed on high fat diet containing half amount of protein from grilled meat supplemented with 5% cinnamon. Subgroups (4) fed on high fat diet containing half amount of protein from grilled meat with 5% green coffee. Subgroups (5) fed on high fat diet containing half amount of protein from grilled meat with 5% cardamom. 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 scarified 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 and liver function as well as antioxidant enzymes were significantly increased ($P<0.05$) compared to the positive control group. The study recommends that intake grilled meat with cinnamon, green coffee, cardamom and their mixture could be beneficial on trial for patients who suffer from obesity.

Keywords---cinnamon, green coffee, cardamom, liver function, 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). Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease in the United States. NAFLD is associated with metabolic disorders, such as type 2 diabetes mellitus, hypertension, dyslipidemia, and obesity. The prevalence of NAFLD is expected to be the leading cause of liver transplantation (Raiya et al., 2018). 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).

Cinnamomum cassia, called Chinese cassia or Chinese cinnamon is an evergreen tree originating in southern China, and widely cultivated there and elsewhere in southern and eastern Asia. It is one of the most important spices and medicinal materials in the world. Cinnamon primarily contains vital oils and other derivatives, such as cinnamaldehyde, cinnamic acid, and cinnamate, and these derivatives play vital roles 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. Coffee is high in not only caffeine but also other bioactive compounds, such as polyphenol and chlorogenic acid that have been suggested to confer diverse health benefits (Ariel et al., 2019). It was determined 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 and cardamom 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, cardamom were obtained from local market. Beef tallow and beef meat were obtained from local market. Adult male albino rats (Sprague- Dawley strain) (n=42 rat) weighing approximately (150±5g.) was 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. The 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 into 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 according to the method of (Chapman et al., 1959).

Biochemical analysis

Serum analyzed to determine the following parameters: Liver function including (AST, ALT and ALP). Determination of serum Aspartate and Alanin amino transferas (AST and ALT). Serum AST and ALT activities were measured according to the method described by (Reitman and Frankel, 1957). Determination of serum Alkaline phosphates (ALP) was determined according to (Roy, 1970). Leptin hormone Leptin was determined according to the methods described by (Zhang et al., 1995).

Antioxidant Enzymes

Serum catalase (CAT) was determined according to (Goth, 1991) while, serum malondialdehyde (MDA) was determined according to (Ermis et al., 2004).

Statistical Analysis

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

Results and Discussion

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

The mean value of Initial body weight (IBW) in the control positive group (control +ve) was increased significantly $p < 0.05$, compared with the mean value of IBW in healthy rats (control -ve group). The data in Table (1) revealed that. the final body weight (FBW) significantly increased ($P < 0.05$) in the (control+ve) compared with the (control -ve group). All treated groups had a significant ($P < 0.05$) decrease in (FBW) compared with (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 this table it could be observed that, significant difference in BWG% between the negative control group (16.10 ± 1.88) fed on basal diet and positive control group (21.67 ± 0.26) fed on high fat diet. The mean value of BWG% of all treating groups which were fed on high protein diet with (5% of cinnamon, 5% of green coffee, 5% of cardamom) and mixture of (cinnamon, green coffee, cardamom) were decreased significantly $p < 0.05$, compared with the positive control group. There were no significant in BWG% between the groups fed on grilled meat with cinnamon or cardamom. The highest wight reduction was observed at the group fed on a grilled meat with a mixture of the tested materials followed by green coffee group.

The data in this Table revealed a decrease in feed intake among the groups treated with the tested materials. From this table it could be observed that, significant ($p > 0.05$) difference in FER between the negative control group fed on basal diet and positive control group fed on high fat diet. The mean value of (FER) of the treating groups which were fed on high protein diet, groups which fed on high protein diet with 5% of either dried cinnamon, green coffee, cardamom or 5% mixture of cinnamon, green coffee, cardamom (one third amount of each herb) were decreased significantly $p < 0.05$, compared with the positive control group. There were no significant in FER between the groups fed on grilled meat with cinnamon or cardamom. The highest weight reduction was observed at the group fed on a grilled meat with a mixture of the tested materials followed by green coffee group. 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, improving weight loss, increasing insulin levels, and reducing fasting blood glucose were also seen in in vitro and in vivo studies (Ranasinghe et al., 2013). Cinnamon can delay gastric emptying, increase glucosidase enzymes and inhibit ATPase of intestinal brush borders; so it can reduce glucose absorption in the small intestine. Furthermore, cinnamon increases glucose transporter 4 (GLUT4), activates glycogen synthase and inhibits glycogen synthase kinase- 3 b. All these actions are decreasing glucose levels and in turn its conversion to glycogen. Methyl Hydroxy chalcone polymers (MHCP) in cinnamon make adipose cells more reactive to insulin by activating the insulin-receptor kinase and inhibiting the insulin receptor-phosphatase, which increases insulin sensitivity and helps increasing body metabolism. Flavonoids and phenolic complexes like epicatechin, catechin, and procyanidin B2 in cinnamon can decrease the absorption of glucose in the intestine, decrease glycogenolysis, and increase glycogen synthesis. Furthermore, these actions can decrease chylomicron absorption. Reducing glucose and chylomicron absorption 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) showed that combined high- protein diet and exercise intervention significantly decreased fat mass and improved lipid profiles, insulin sensitivity, glucose tolerance, and inflammation in middle-aged adults with obesity. Also, the results are in the line with (Joohee and Hyun-Sook, 2022) who showed that rats fed on normal diet with 1% cinnamon extracts or fed on high-fat diet with 1% cinnamon extracts had lower final body weight and body weight gain than normal diet group and high-fat-diet group. 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 that 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 and regulate blood glucose by several mechanisms. Moreover, Cimi et al., (2020) reported that low dose green coffee extract (GCE) has a beneficial effect on body weight. Our findings strengthen the scientific evidence on the property of GCE in the management of obesity and hyperlipidemia. Green coffee beans are valued as functional ingredients with several health benefits including weight loss (Roshan et al., 2018). In a randomized placebo controlled clinical trial, we have evaluated the effect of a 12-week ingestion of 500 mg/day CGA-7, on the primary outcome measures such as body weight, BMI, lean mass/fat mass ratio and body fat percentage in overweight subjects. This study was conducted to scientifically validate the weight loss properties of a standardized green coffee bean extract containing seven isomers of CGAs. Here we have documented the efficacy of CGA-7 in reducing the risk of obesity among the healthy overweight individuals (Sudeep and Shyam., 2021).

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 and diabetes genes expression in Polycystic ovary syndrome (PCOS) women. Fatemeh et al., (2017) observed that after 2 months intervention with three-gram cardamom, weight, BMI, WC, insulin sensitivity was significantly decreased compared to control group, however, other glycemic indices including FBS, insulin, and HOMA-IR had not changed after intervention. Another trial by Aghasi et al., (2019) showed that Glycated hemoglobin (HbA1c), insulin, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) were significantly decreased after green cardamom supplementation. Since, we gave both groups the low calorie diet for ethical consideration, therefore, it seems changes in anthropometric indices after intervention in both groups are normal. Green cardamom is rich in flavonoids and isoflavones which are contributed in reducing insulin resistance by decreasing adipose tissue storage.

Table 1
The effect of high protein die (grilled meat) with cinnamon, green coffee, cardamom and their mixture on bodyweight 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
Grilled meat		205.36±3.44 ^a	176.50±1.32 ^c	-14.02±0.80 ^c	15.00	-0.043±0.03 ^b
Grilled meatwith 5%cinnamon		202.23±2.38 ^a	160.93±2.69 ^e	-20.43±0.52 ^d	13.50	-0.068±0.01 ^c
Grilled meat with5%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 with 5% 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 theirmixture		204.66±2.02 ^a	137.36±1.33 ^g	-32.76±0.70 ^f	12.20	-0.122±0.03 ^e

BWG%: Body weight gain %.

All results are expressed as mean \pm SE.

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

IBW = Initial Body Weight, FBW = Final Body Weight

The effect of high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture on serum liver function of obese rats

As seen in Table (2) serum concentrations of (AST), (ALT) and (ALP) were elevated significantly ($P < 0.05$) in the positive control group compared with negative control group. all treated groups were decreased significantly $p > 0.05$ as compared to high protein diet. the highest decreased was observed at the group that fed on high protein diet with the mixture of (cinnamon, green coffee, cardamom) then the group fed on high protein diet (grilled meat) with cinnamon, green coffee and cardamom respectively. Parivash et al., (2020) reported that cinnamon supplementation had no significant effect on liver enzymes in adults. However, the effect of cinnamon on ALT levels was significant at the dosages of < 1500 mg/day, in trials lasting > 12 weeks, and in trials conducted of both genders. The exact mechanism of cinnamon on the liver function is still unclear. However, previous studies showed that cinnamon can inhibit liver damage via peroxisome proliferator activated receptor-gamma (PPAR γ) (Rau et al., 2006). Inactivation of PPAR in hepatocytes is associated with an increase in body and liver weight, as well as a decrease in serum AST levels. (Mirzaii et al., 2016) Moreover, PPAR γ activation is associated with other beneficial effects such as down-regulation of the expression of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and C-reactive protein (CRP), which involved in the "second hit" of the pathogenesis of NAFLD. (Sahebkar., 2011). In addition, reports have indicated that oxidative stress and immune system disorder plays important roles in contribute to liver dysfunction such as nonalcoholic fatty liver disease NAFLD. (Chen., 2020).

Milad et al., (2019) Green cardamom (GC) supplementation in overweight or obese nonalcoholic fatty liver disease (NAFLD) patients showed a significant beneficial effect on the grade of fatty liver, serum glucose indices, and lipid profiles. The proposed various mechanisms of GC effect on glucose and lipid profiles include antioxidant capacity increment (Asimi et al., 2016). inhibition of inflammation (Rahman et al., 2017). improvement of obesity, enhanced insulin activity and sensitivity (increased glycogenesis, decreased gluconeogenesis) (Alshammari, 2017). increased expression and activity of PPAR γ (improved glycemic control) (Bhat et al., 2015), and inhibition of cholesterol synthesis (Darwish and Abd, 2013). The oxidative stress may damage tissue and impair insulin secretion and glucose transmission (Rahman et al., 2017). Therefore, reducing oxidative stress can be effective in improving glucose metabolism. The hypolipidemic effect of GC may also improve plasma glucose and insulin levels and enhance insulin function (Aboelnaga, 2015) The effect of flavonoids on glycemic indices is related to reduced glucose absorption and enhanced glucose tolerance (Rahman et al., 2017).

Jeddiah and Patrick, (2019) suggest that decreasing protein consumption may be one simple strategy to decrease blood ammonia levels and minimize the risk of developing hepatic encephalopathy for many liver disease patients. Paola et al., (2019) showed that coffee consumption reduces HFD-induced liver damage by modulating pathways involved in the gut–liver axis. Reducing hepatic fat deposition possibly through increased fat oxidation in the liver; Increasing cholesterol intestinal efflux; Reducing lipid digestion and ameliorating the intestinal system involved in lipid sensing and energy metabolism regulation, and Ameliorating gut barrier function through a restoration of tight junction proteins in the duodenum and colon. Coffee consumption reduced high-fat diet (HFD)-induced liver steatosis and circulating ALT, which is consistent with our previous findings (Vitaglione et al., 2010). The reduced fat deposition (as demonstrated by histopathology and supported by the reduced liver:body weight ratio) and inflammation in the liver could be a consequence of an increased fat oxidation (Souza-Mello, 2015) which was supported by the up-regulation of PPAR- α . This agrees with other studies demonstrating the ability of polyphenols, as pure compounds or as part of foods, to induce lipid oxidation in the liver of animals fed with an HFD (Salomone et al., 2016).

Table 2
Effect of high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture on serum AST, ALT and ALP of obese rats

Parameters Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control (-ve)	59.87±1.49f	26.53±0.45d	70.16±1.63f
Control (+ve)	100.86±2.25a	40.40±1.34a	104.33±1.16a
Grilled meat	94.11±0.40b	36.01±0.99b	94.95±1.39b
Grilled meat with 5%cinnamon	73.87±1.23d	29.76±0.49cd	80.63±2.60de
Grilled meat with 5%green coffee	81.60±0.92c	32.49±1.05bc	86.66±2.49cd
Grilledmeat with 5%cardamom	85.06±1.54c	34.76±1.35b	89.21±1.84bc
Grilled meat and their Mixture	65.17±1.85e	29.76±1.77cd	77.31±3.51e

All results are expressed as mean \pm SE.

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

Serum leptin

The concentrations of serum leptin was recorded in Table (3) the results indicated that leptin levels in the positive control group significantly increased ($P < 0.05$) compared to normal group. While all treated groups with high protein diet (grilled meat) and groups with high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture had significant ($P < 0.05$) decrease compared with the positive control group. all treated groups were decreased significantly $p > 0.05$ compared with high protein diet the highest decreased was observed at the group that fed on high protein diet with the mixture of (cinnamon, green

coffee, cardamom) then the group fed on high protein diet (grilled meat) with cinnamon, green coffee and cardamom respectively. the highest improvement for leptin levels was observed in the group fed on high protein diet (grilled meat) with the mixture of (cinnamon, green coffee, cardamom) which is close to the negative control.

Andrea et al., (2019) reported that Leptin, a hormone that is capable of effectively reducing food intake and body weight, 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. Friedman, (2011) mentioned that leptin is a peptide hormone secreted by adipose tissue in proportion to its mass. When leptin circulates in blood and acts on the brain to regulate food intake (appetite) and energy expenditure. When body fat mass decreases, the plasma leptin levels decreases so stimulating appetite and suppressing energy expenditure till fat mass is restored. 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 prevents the conversion of androgens 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).

Table 3

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

Parameters	Leptin hormone (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 ^c
Grilled meat with 5%green coffee	22.72±0.99 ^d
Grilledmeat with 5%cardamom	26.24±0.74 ^c

Grilled meat and their mixture	15.43±0.89 ^f
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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 CAT and MDA of obese rats

Data of CAT was recorded in Table (4), The results indicated that CAT levels in the positive control group significantly decreased ($P < 0.05$) compared to normal group. While all treated groups with high protein diet (grilled meat) and groups with high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture had significant ($P < 0.05$) increase compared with the positive control group. all treated groups were increased significantly $p > 0.05$ compared with high protein diet. the highest increase was observed at the group that fed on high protein diet with the mixture of (cinnamon, green coffee, cardamom) then the group fed on high protein diet (grilled meat) with cinnamon, green coffee and cardamom respectively. Data of MDA was recorded in Table (4) The results indicated that MDA levels in the positive control group significantly increased ($P < 0.05$) compared to normal group. While all treated groups with high protein diet (grilled meat) and groups with high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture had significant ($P < 0.05$) decrease compared with the positive control group. all treated groups were decreased significantly $p > 0.05$ compared with high protein diet, the highest decreased was observed at the group that fed on high protein diet with the mixture of (cinnamon, green coffee, cardamom) then the group fed on high protein diet (grilled meat) with cinnamon, cardamom and green coffee respectively.

Ebtihal, (2016) concluded that feeding with Arabic coffee and green coffee had contained the highest amounts in antioxidant and total phenolic acid and it was improved lipid profile parameters and level of leptin hormone. From the obviously results, it could be recommended that the Arabic and green coffee used for to improve weight serum lipids and leptin hormone. Haddad et al., (2021) showed that consumption of green coffee methanolic extract accompanied by silymarin caused a suppressive effect on oxidative stress and confirmed authors' previous reports concerning the antioxidant activity of green coffee and its ability to trap hydroxyl radicals and superoxide anions. Haddad et al., (2021) also showed that the antioxidative effect of green coffee methanolic extract accompanied by silymarin on enzymatic antioxidant systems was reflected by increased concentrations of antioxidants (TAC, GSH, GST, CAT and SOD) and a decreased lipid peroxidation that may be ascribed to its richness in caffeine, which is considered as an important component of coffee. Moreover, the antioxidant activity of green coffee makes it able to trap hydroxyl radicals or superoxide anions because of its content of both phenolic compounds and chlorogenic acid (Morishita and Ohnishi, 2001).

Haddad et al., (2021) observation that the consumption of green coffee methanolic extract escorted by silymarin had a protective effect on oxidative stress (MDA) and

antioxidant redox system. Also showed that consumption of green coffee methanolic extract accompanied by silymarin causes a suppressive effect on oxidative stress and confirmed the findings of other authors showing the antioxidant power of green coffee (Abdelaal et al., 2019). Furthermore, oxidative DNA damage was decreased; and glutathione level and glutathione reductase activities were increased after consumption of green coffee. Cinnamon extracts have been shown to be useful in decreasing fasting plasma glucose, cholesterol, and triglycerides in diabetic patients (Khan et al., 2003). Similarly, application of cinnamon extract reduced liver MDA levels in carbon tetrachloride-poisoned rats and improved SOD, CAT, and GSHPx activities (Moselhy and Ali, 2009). Cinnamon has been shown to prevent hyperlipidemia and improved glucose tolerance in rats fed fructose/high fat (Couturier et al., 2011).

Tuzcu et al., (2011) showed similar reductions in Nrf2 and HO-1 expressions as increased serum MDA in HFD-fed rats. In addition, cinnamaldehyde, an important flavor component in cinnamon essential oil upregulated Nrf2 expression, stimulated its translocation to the nucleus, and increased HO-1, NQO1, CAT, and GPx1 expression under high glucose conditions (Wang et al., 2015). Wondrak et al., (2010) reported that cinnamaldehyde and cinnamon extract upregulated cellular protein levels of Nrf2 in human colon cancer cells and recognized Nrf2 targets involved in the antioxidant response including HO-1 and gamma-glutamyl cysteine synthetase. Oxidative stress is a major cause of different complications originating from obesity (Feillet-Coudrayetal, 2009). Aroor and DeMarco, (2014) found that concentration of MDA, NO and APOP which are indices of oxidative stress were significantly elevated in liver of HCHF administered rats, and treatment with cardamom reduces this marker compare with HCHF rats. The level of antioxidant enzymes like catalase, and SOD activities and GSH concentration in the liver of HCHF diet fed rats were seen significantly lower than control group which signifies that obesity has reduced the antioxidant capacity of the liver cells Cardamom supplementation restored these enzymes activities in HCHF diet fed rats, which indicates that cardamom improve the antioxidant capacity by increasing antioxidant enzymes.

Table 4

Effect of high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture on CAT and MDA of obese rats

Parameters	CAT (u/ml)	MDA (umol/dl)
Control (-ve)	129.96±1.00 ^a	2.31±0.11 ^c
Control (+ve)	73.80±1.64 ^f	6.51±0.38 ^a
Grilled meat	92.17±1.99 ^e	4.44±0.27 ^b
Grilled meat with 5%cinnamon	119.76±0.75 ^b	3.01±0.12 ^c
Grilled meat with 5%green coffee	114.60±1.88 ^c	3.91±0.03 ^b
Grilledmeat with 5%cardamom	108.26±1.15 ^d	3.89±0.11 ^b
Grilled meat and their Mixture	123.33±1.11 ^b	2.70±0.12 ^{de}

All results are expressed as mean \pm SE. Values in each column which have different letters are significantly different ($p < 0.05$). In conclusions, high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture may be beneficial for patients who suffer from obesity. specially at the group that fed on high protein diet with the mixture of (cinnamon, green coffee, cardamom) by the reduction the mean value of on serum AST, ALT, ALP, serum leptin and MDA levels and causing an increase on the value of CAT levels. So that, it could be suggested that, high protein diet (grilled meat) with cinnamon, green coffee, cardamom and their mixture could be used as a suitable therapy for obesity.

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