

## Biochemical and nutritional studies on cocoa powder and its effect on blood lipids in experimental rats

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### ABSTRACT

Cocoa powder considered a high rich source of polyphenols so it reduce the lipid profile in blood and the risk of cardiovascular disease. The present investigation aimed to assess the biochemical and nutritional properties on cocoa powder and its effect on blood lipids in rats. Twenty Four male albino rats Sprague Dawley Strain weighing (150±10 gm.) used in experiment. All rats were fed on basal diet (one week) for adaptation. Then randomly divided into two main groups as following : The first main group include: (G1) fed on basal diet as a control negative group (-ve). The second main group was fed on high fat diet (HFD) for (4weeks) to induce hyperlipidemia. Then, rats were divided into three groups as following: (G2) fed on high fat diet as a positive control group (+ve), (G3,G4), were fed high fat diet supplemented with cocoa powder (5%, 10% respectively). The results indicated a significant decrease in BW, FI, and FER in rats fed with basal diet + cocoa powder nearly from negative control group (-ve). (HFD) rats showed a significant increase in serum total cholesterol, triglycerids, LDL-c, VLDL-c, compared to negative control group(-ve). On the other hand, (HFD) rats showed a significant decrease in serum high-density lipoproteins (HDL-c) compared to normal control group (-ve). While cocoa powder improve lipids profile and protective from cardiovascular risk .

**Keywords:** Cocoa powder, Hyperlipidemia, total cholesterol, triglycerids and lipoproteins.

### INTRODUCTION

Atherosclerosis is considered as a low- grade chronic inflammatory process resulting from interaction between plasma lipoprotein, cellular components and the extracellular matrix of the arterial wall (Tedgui and Mallat , 2006). Cardiovascular disease (CVD), as a group, is a leading cause of death in the United States and worldwide, causing over 16.7 million deaths globally in 2002. (Mackay and Mensah, 2004). Atherosclerosis is crucial to (CVD ) and is strongly related to dyslipidemia. Classically, dyslipidemia includes high total cholesterol, high low-density lipoprotein (LDL) cholesterol, low high-density lipoprotein (HDL) cholesterol and high triglycerides (Abuzaid and Al-Menyar, 2015). Studies suggest CVD diseases may be preventable by lifestyle modification, such as exercise and nutrition (Tanasescu *et al.*, 2002; Weisburger, 2000; Stampfer *et al.*, 2000).

Nowadays, there is growing interest in the use of plant foods for the prevention and management of CVD and other related disorders, with a special emphasis on cocoa and its products (Arranz *et al.*, 2013). Fruits, vegetables, tea and chocolate are major sources of antioxidants, flavonoids , which have been shown to have protective effects against CDV (Eyre *et al.*, 2004; Goldstein *et al.* , 2001) . Cocoa products contain greater antioxidant capacity and greater amount of flavonoids per serving than all others sources, it is important to explore chocolate's potential effects on CVD (Baltimore *et al.*, 1996, Kris-Etherton and Keen, 2002). Cocoa (*Theobroma cacao* L.) is one of the most ancient cultivated human crops. It is originated in Mexico and is central to the local diet (Colombo *et al.*, 2012). Consumption of cocoa is related to higher-quality diets, including higher intakes of protein, antioxidants and a number of vitamins and mineral elements. Cocoa

intake is also associated with several beneficial health effects, particularly reduced risks of obesity, diabetes, hypertension and CVD (Sentürk and Günay, 2015; Hooper *et al.*, 2012). Cocoa bean is loaded with the polyphenols such as quercetin (including its glucoside), clovamide, deoxyclovamide and procyanidin, Epicatechin, catechin) (Sanbongi *et al.*, 1998; Hammerstone, 1999 ). Research indicates that the flavonoids, a class of polyphenols, has antioxidant characteristics with potential health benefits that may reduce the risk of cardiovascular disease and cancer (Allen *et al.*, 2008). The specific antioxidants in chocolate (i.e., cocoa flavanols) include catechin and epicatechin, which are single flavanol molecules structurally similar to the antioxidants found in grapes and tea (Arts and Hollman, 2005).

Cocoa also appears to have anti-aging and anti-inflammatory properties. Cocoa is a good source of the minerals magnesium, sulphur, calcium, iron, zinc, copper, potassium, and manganese; plus some of the B Vitamins. Cocoa enhanced clot prevention afforded by cocoa flavanols (Rein *et al.*, 2000 ).

In general, most of the nutritional and clinical studies linking cocoa with CVD have been mainly devoted to the effects of bioactive components such as polyphenols and flavonoids, and have often paid little or no attention to the cocoa fat as a possible mechanism for explaining such link. In fact, fats are the highest variable components of the diet both in quantitative and in qualitative terms, and they are the most relevant dietary factor affecting serum lipids, especially in cases with dyslipidemia (Galli, 2012). Nevertheless, controlled human or animal studies that link consumption of defatted cocoa and cholesterol with serum lipids and lipoproteins in particular are generally lacking.

Previous studies have suggested that dark chocolate consumption reduces blood pressure (Grassi *et al.*, 2005b;

Grassi *et al.*, 2008), improves insulin sensitivity as shown by significantly higher QUICKI (quantitative insulin sensitivity check index) measurements (Grassi *et al.*, 2008), improves vascular endothelial function and reverses vascular dysfunction (Engler *et al.*, 2004; Grassi *et al.*, 2005; Wang-Polagruto *et al.*, 2006), reduces insulin resistance as evidenced by significantly lower HOMA-IR (homeostasis model assessment of insulin resistance) (Grassi *et al.*, 2005).

The present investigation aimed to assess the improvement role of cocoa powder on body weight and biochemical parameters of hyperlipidemic rats.

## MATERIALS AND METHODS:

### Materials:

-Cocoa powder : was purchased from local market Tanta City, Elgharbia Governorate, Egypt.

- Casein, choline chloride and DL-methionine, vitamins and salt mixture were obtained from El -Sharkiya Co., Sun flower oil and corn starch also were obtained.

Animals: Male albino rats (n = 24) of Sprague Dewey Strain weighting (150 ±10g gm) were obtained from the animal colony, Helwan farm, Vaccine and Immunity Organization, Ministry of Health, Helwan Governorate, Egypt. Rats were kept in single wire cages with wire bottoms under hygienic conditions. The diet was introduced to the rats in special food containers to avoid scattering of food. Also water supply was given ad-libitum and check daily. The rats fed basil diet for one week as adaption period .Standard diet was prepared from fine ingredients per 100g.

- Kits were purchased from Egyptian American Company for Laboratory Service and Supplied by Alkan Company.

### Methods :

#### Chemical analysis:

Cocoa powder was subjected to chemical analysis to determine: Total phenols;

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phenolic compounds were determined by HPLC according to method of Goupy *et al.* (1999) at central lab of food technology research Institute Agric. Res. Cent. Egypt.

### Experiment design:

Twenty four male Sprague-Dawley Albino rats( 150+ 10 gm ) were acclimatized for one week, fed basal diet according to Reeves *et al.*,(1993) and water *ad libitum*. Rats divided into two main groups as following:

- The first main group (G1): rats fed on basal diet as a control negative group (v-).
- The second main group was fed on high fat diet (HFD) for (4 weeks) to induce hyperlipidemia. Then, rats were divided into three groups as following:
  - (G2) fed on high fat diet as a positive control group (v+),
  - (G3, G4) fed high fat diet supplemented with cocoa powder (5% , 10% , respectively).

At the end of experiment (28) days, rats were be deprived of food and water overnight before being sacrificed. Blood samples were collected in dry centrifuge tubs from hepatic portal veins. Serum samples were separated by centrifugation at 4000 rpm for 10 minutes and kept in plastic vial at -20 till analysis.

### - Determination of some Biological parameters:

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 determined according to Champman *et al.* (1959).

### -Biochemical analysis of serum:

The serum samples was separated to estimate some biochemical parameters, i.e. total cholesterol and Triglycerides according to Fossati and Pranciopl (1982). Very Low Density Lipoprotein Cholesterol (VLDL – c) according to the equation of Friedewald *et al.* (1972). HDL cholesterol was determined according to the method

described by Burstein 2017), the concentration of LDL was estimated according to the equation of Friedewald *et al.* (1972 ).

### Statistical analysis:

The obtained data was statistically analyzed by SPSS computer software. The calculated was occurred by analysis of variance ANOVA and follow up test LSD by SPSS according to Armitage and Berry (1987).

## RESULTS

The macronutrients composition of cocoa powders used in this study is given in table I. cocoa powder was found to contain low content of fat (13.7 g/100), high content of carbohydrates ( 57.9 g).The amount of protein is ( 16.6 g). Also, cocoa is a good source of the minerals magnesium, sulphur, calcium, iron, zinc, copper, potassium, and manganese; plus some of the B Vitamins.

**Table 1: Nutrition consumption of cocoa powder ( per 100 gram ):**

Parameter	Kcal / Gm
Calories (Kcal)	400
Total carbohydrates (g)	57.9
Starch (g)	6.4
Dietary fiber (g)	33.2
Total fat (g)	13.7
SFA (g)	8.1
MUFA (g)	4.6
PUFA (g)	0.4
Cholesterol (mg)	0.0
Proteins (g)	19.6
magnesium	125 %
calcium	13 %
iron	77 %
zinc	45 %
copper	189 %
potassium	44 %
manganese	192 %

The total phenolic and total proanthocyanidin content of the cocoa powder was determined by Bathee Smith methods (Andres-Lacueva *et al.*, 2013). Singletonand Rossi, 2000). Individualized

phenolic compounds were determined by HPL-c analysis of cocoa powder. The major Individualized phenolic components are Epicatechin (46.08) followed by Procyanidin B2 (36.54mg/g) .While the lowest compounds are Quercetin-3-glucuronide (0.10). Cocoa powder contains high amounts of total polyphenols (495.2mg/g ) and total proanthocyanidins (425.7mg/g).

**Table (2): Phenolic compounds in cocoa powder (ppm).**

Flavans (mg/ g)	Mean value
Catechin	10.41
Epicatechin	46.08
Procyanidin B2	36.54
Isoquercetrin	2.23
Quercetin	0.22
Quercetin-3-arabioside	0.70
Quercetin-3-glucuronide	0.10
Total polyphenols	495.2
Total proanthocyanidins	425.7

**Table 3: Mean values  $\pm$  SD of feed intake (g/d) and BWG% of control rats and treated groups (n= 24)**

Group \ Parameter	Feed intake	BWG%
Normal control (-ve )	15.67 $\pm$ 1.0 <sup>c</sup>	15.3 $\pm$ 1.09 <sup>a</sup>
Positive control (+ve)	14.27 $\pm$ 1.0 <sup>a</sup>	35.15 $\pm$ 2.7 <sup>c</sup>
HFD + 5% cocoa	14.39 $\pm$ 2.0 <sup>b</sup>	26.97 $\pm$ 1.8 <sup>b</sup>
HFD + 10% cocoa	14.46 $\pm$ 1.0 <sup>c</sup>	25.8 $\pm$ 1.05 <sup>b</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

#### Organs Weight / Body Weight % :

Data presented in Table (3) showed the mean value of liver weight / body weight % of all treated groups had no significant differences ( $P > 0.05$ ) as compared to the value of the control - ve group. The lowest mean value of liver weight / body weight % was recorded for the control +ve, while the highest mean value was recorded for group of rats treated with cocoa powder (5 %).

#### Biological evaluation

Data presented in Table (3) showed the effect of cocoa powder on feed intake (FI) and body weight gain (BWG%) in hyperlipidemic rats . The results revealed that rats reserved supplemented diet with cocoa powder demonstrated no significant  $p > 0,05$  in the mean value of feed intake as compared to negative control (-ve). Positive control group (+ve) recorded significant ( $P > 0.05$  ) increase in BWG% comparing to negative control group (-ve). While, rats reserved supplemented diet with cocoa powder showed clear significant ( $P > 0.05$  ) decrease in BWG % compared to the positive control group (+ve).

As for the mean value of kidney weight / body weight %, the lowest mean value was recorded for rats treated with cocoa powder (10 %) while the highest mean value was recorded for the control - ve group. Regarding spleen weight / body weight rats treated with 5% or 10% recorded significant decrease in mean value compared to the value of the control + ve group, and closed with control -ve group.

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**Table 2: Mean values  $\pm$  SD of organs weight / body weight % of control rats and treated groups (n= 36).**

Parameter Group	Liver	kidney	spleen
Normal control ( v-)	4.38 $\pm$ 3.8 <sup>a</sup>	1.56 $\pm$ 0.31 <sup>a</sup>	0.41 $\pm$ 0.22 <sup>a</sup>
Positive control ( v+)	3.4 $\pm$ 0.9 <sup>b</sup>	1.52 $\pm$ 0.38 <sup>ab</sup>	0.51 $\pm$ 0.16 <sup>b</sup>
HFD + 5% cocoa	4.05 $\pm$ 0.79 <sup>a</sup>	1.32 $\pm$ 0.13 <sup>bc</sup>	0.46 $\pm$ 0.19 <sup>a</sup>
HFD + 10% cocoa	3.8 $\pm$ 0.72 <sup>b</sup>	1.04 $\pm$ 0.080 <sup>c</sup>	0.45 $\pm$ 0.18 <sup>a</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. - Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

### Biochemical Analysis :

#### Serum Cholesterol and Triglycerides :

As shown in Table (4) the mean value of serum (cholesterol and triglycerides) for the -ve control group was significantly decreased as compared to the control +ve . It could be observed that, rats reserved supplemented diet with

(cocoa powder 5% or 10% ) recorded significant ( $P > 0.05$ ) decrease in the mean value of cholesterol and triglycerides compared to the control +ve group. The best results of the mean values  $\pm$ SD of cholesterol were recorded for rats reserved supplemented diet with (cocoa powder 10% ).

**Table 3: Mean values  $\pm$  SD of serum cholesterol and triglycerides of control rats and treated groups (n= 36)**

Parameter Group	Total Cholesterol( TC)	Triglycerides(TG)
Normal control ( v-)	128.18 $\pm$ 2.2 <sup>ab</sup>	135.2 $\pm$ 3.4 <sup>c</sup>
Positive control ( v+)	195.79 $\pm$ 12.9 <sup>b</sup>	201.8 $\pm$ 5.8 <sup>a</sup>
HFD + 5% cocoa	131.1 $\pm$ 3.2 <sup>a</sup>	147.07 $\pm$ 4.4 <sup>ab</sup>
HFD + 10% cocoa	129.1 $\pm$ 2.1 <sup>b</sup>	138.4 $\pm$ 4.6 <sup>b</sup>

Significance is expressed at  $p < 0.05$  using one way ANOVA test. - Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

#### Serum Lipoprotein:

It could be noticed from data in Table (4) that the mean value of HDL-c of control +ve group was significantly decreased ( $P < 0.05$ ) as compared to control -ve group. However, the mean value ( $\pm$ SD) of LDL and VLDL- in +ve group increased significantly ( $P < 0.05$ ) as compared to control -ve and cocoa powder groups. The best result was recorded for rats fed on cocoa powder (10%) which

closed to the mean value of control -ve group. Concerning the mean value ( $\pm$ SD) of LDL-c it was noticed that Control +ve group significantly increased ( $P < 0.05$ ) as compared to the control -ve and other cocoa powder groups. The best result was recorded for rats fed on cocoa powder (10%).

Results also showed no significant differences ( $P < 0.05$ ) among cocoa powder groups in the mean value of VLDL.

**Table 4: Mean values ( $\pm$ SD) of serum lipoproteins of control rats and treated groups (n= 36)**

<b>Parameter</b> <b>Group</b>	<b>HDL</b>	<b>LDL</b>	<b>VLDL</b>
<b>Normal control (v-)</b>	83.08 $\pm$ 12.9 <sup>a</sup>	76.8 $\pm$ 8.5 <sup>b</sup>	33.03 $\pm$ 1.09 <sup>b</sup>
<b>Positive control (v+)</b>	59.4 $\pm$ 11.6 <sup>b</sup>	101.92 $\pm$ 5.1 <sup>c</sup>	40.37 $\pm$ 1.15 <sup>a</sup>
<b>HFD + 5% cocoa</b>	77.1 $\pm$ 3.2 <sup>c</sup>	78.07 $\pm$ 2.6 <sup>ab</sup>	36.33 $\pm$ 2.6 <sup>ab</sup>
<b>HFD + 10% cocoa</b>	80.36 $\pm$ 9.1 <sup>a</sup>	74.71 $\pm$ 2.1 <sup>b</sup>	35.6 $\pm$ 0.92 <sup>ab</sup>

- Significance is expressed at  $p < 0.05$  using one way ANOVA test.

- Values which have different letters in each column differ significantly, while the difference among those with similar letters completely or partially is not significant.

### DISCUSSION

The observed reduction in LDL and TC levels may be attributable to flavonoids contained in cocoa and dark chocolate. Flavanols in cocoa are present as monomers, oligomers or polymers, better known as procyanidins, and generally are thought to inhibit cholesterol absorption as well as the expression of LDL cholesterol receptors (Matsui *et al.*, 2005). The results are in agreement with Min *et al.* (2013) who demonstrated that consumption of high fat diet significantly increased body weight while consumption of cocoa powder significant decrease in body weight and induced body loss.

The present results are in line with Kollar *et al.* (2002) found that flavonoids significantly reduce the value of total cholesterol in the serum of hypercholesterolemic rats. However, relative variability in the nutritional properties of cocoa powder has been reported. This variability may be attributed to a number of factors, such as differences in genotype, maturity stage, postharvest handling and storage conditions, product quality and analytical procedures (Baba *et al.*, 2007). This observed reduction may be attributable to flavonoids contained in cocoa and dark chocolate. Flavan-3-ols in cocoa are present as monomers, oligomers or polymers, better known as procyanidins, and generally are thought to inhibit cholesterol absorption as well as the

expression of LDL cholesterol receptors (Matsui *et al.*, 2005).

The presently obtained fat and energy values for defatted cocoa were consistent with those reported elsewhere (Wan *et al.*, 2001). Consistently, in this study, chocolate feeding increased serum HDL-cholesterol and decreased triglycerides. Cholesterol had some increasing effect on total cholesterol and VLDL-cholesterol, but this effect did not reach statistical significance. In line with these results, serum total cholesterol has been shown to increase or remain unchanged as a result of cholesterol feeding in animals. Nicod *et al.* (2014) found that polyphenols from cocoa administered at a dietary dose moderately modulate intestinal inflammation but do not increase cholesterol secretion by intestinal cells or enhance HDL functionality.

Other studies in animal models of obesity and diabetes demonstrate the effects of cocoa extract in reducing serum and hepatic triglycerides. In a diet-induced obesity model of Wistar rats, ten weeks of cocoa extract supplementation (14 and 140 mg per kg body mass per day) revealed a significant decrease in serum and hepatic triglyceride content. Indeed, some human studies showed that administration of polyphenol-rich foods such as cocoa powder modulated and decreased LDL-C and increased HDL-C concentrations (Do, 2010).

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A meta-analysis showed that short-term polyphenol-rich cocoa consumption (duration of intervention ranging from 14 to 126 days) decreased LDL-C by approximately 2.98 mg/dL and increased HDL-C by 1.78 mg/dL (Galli, 2012). Basu *et al.* (2015) reported conventional lipid data from the present clinical trial showing that polyphenol-rich cocoa increased postprandial HDL-C. Crew *et al.* (2008) indicated that regular ingestion of dark chocolate may have no adverse effects on serum lipid profile, whereas others have suggested that intake of dark chocolate reduced serum LDL cholesterol and triglyceride (TG) levels (Engler *et al.*, 2004; Grassi *et al.*, 2005), and increased serum high-density lipoprotein (HDL) cholesterol measurements (Mursu *et al.*, 2004).

Polyphenol-rich cocoa may have attenuated the postprandial rise in VLDL and chylomicron particles by interfering with their synthesis, which is typically increased in the presence of insulin resistance. Also, catechins have been shown to inhibit intestinal lipid absorption in animal and epidemiological studies (Lee *et al.*, 2003). Considering these clinical trials and the evidence in the present work, we contend that polyphenol-rich cocoa conferred cardio-protection by sustaining HDL concentrations and HDL concentrations for 6 h after a fast-food-style HF meal.

### CONCLUSIONS

The present study indicated that consumption of cocoa powder and dark chocolate improved fraction lipid (total cholesterol, triglycerides and high density lipoprotein HDL) while lowered the mean value of body weight and LDL, VLDL. Therefore one can intake cocoa to decrease the risk of hyperlipidemia.

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### دراسات كميوجيوية وتغذوية علي مسحوق الكاكاو وتأثيره علي دهون الدم في جردان التجارب

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#### المستخلص

يعتبر مسحوق الكاكاو من المصادر الغنية بالفينولات العديدة لذلك فهو يقلل من نسبة الدهون في الدم وخطر الإصابة بأمراض القلب والأوعية الدموية. هدفت الدراسة الحالية إلى تقييم الخصائص الكميوجيوية والتغذوية لمسحوق الكاكاو وتأثيره علي الفئران المصابة بفرط دهون الدم. أستخدم في هذه الدراسة أربع وعشرون ذكر من جردان ألبينو وزنها (150 ± 10 جم). تم تغذية جميع الفئران على النظام الغذائي الأساسي (أسبوع واحد) للتكيف. ثم قسمت عشوائياً إلى مجموعتين رئيسيتين على النحو التالي: المجموعة الرئيسية الأولى تشمل: (مج 1) تتغذى على النظام الغذائي الأساسي كمجموعة ضابطة سلبية (-V). تم تغذية المجموعة الرئيسية الثانية على نظام غذائي عالي الدهون (HFD) لمدة (4 أسابيع) للتحث على ارتفاع نسبة الدهون في الدم. ثم قسمت الفئران إلى ثلاث مجموعات على النحو التالي: (مج 2) تغذت على حمية غنية بالدهون كمجموعة ضابطة إيجابية (+ve) ، ومجموعتين (مج 3 ، مج 4) تم تغذيتها على نظام غذائي غني بالدهون مكمل بمسحوق الكاكاو (5%، 10% على التوالي).

أشارت النتائج إلى انخفاض معنوي في وزن الجسم و المأخوذ الغذائي في الفئران التي تم تغذيتها على مسحوق الكاكاو مقارنة بالمجموعة الضابطة السلبية (-V). أظهرت الفئران عالية الدهون في الدم زيادة معنوية في الكوليسترول الكلي في الدم، والدهون الثلاثية، البروتينات الدهنية منخفضة الكثافة، البروتينات الدهنية منخفضة الكثافة جدا ، مقارنة بالمجموعة الضابطة السلبية (-V). من ناحية أخرى ، أظهرت الفئران عالية الدهون في الدم انخفاضاً معنوياً في البروتينات الدهنية عالية الكثافة) مقارنة بالمجموعة الضابطة السلبية (-V). بينما عمل مسحوق الكاكاو على تحسين مستوي الدهون المفيدة والحماية من مخاطر الإصابة بأمراض القلب والأوعية الدموية.