The Effect of Cocoa Powder, White Chocolate and Dark Chocolate on Oxidative Stress and Lipid Profile on Hypercholesterolemic Rats

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Abstract: Aim of the work: Assessing the effect of cocoa powder, white chocolate and dark chocolate on oxidative stress and lipid profile in hypercholesterolemic rats **Methods**: 48 male albino rats were divided into two separate experimental designs, the first experiment consists of 4 groups of 8 rats each including group 1: control group fed basal diet (BD), group 2: high fat high cholesterol group (HFC), group 3 and 4 : HFC +5% and 10% cocoa powder respectively for 4 weeks. The second experiment consists of 4 groups of 8 rats each including group 1: control group fed basal diet (BD), group 2: high fat high cholesterol group (HFC), group 3 and 4: HFC +2% white and dark chocolate respectively for 4 weeks. **Results:** (HFC) fed diet rats showed a significant increase in serum total cholesterol, triacylglycerol, LDL-C, MDA and atherogenic index, compared to (BD) group. On the other hand, (HFC) fed diet rats showed a significant decrease in serum high-density lipoproteins (HDL), total protein, erythrocyte Superoxide dismutase (SOD) and reduced glutathione (GSH) compared to healthy control rats. Consumption of cocoa powder or dark chocolate by hypercholesterolemic rats resulted in a significantly decrement in lipid parameters and improvement in antioxidant status as compared to hypercholesterolemic rats. **Conclusion:** The results suggest that cocoa powder or dark chocolate had Hypolipidemic and antioxidant effect, which may be attributable to flavonoids contained in cocoa and dark chocolate.
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1. Introduction

Oxidative stress and the subsequent accumulation of reactive oxygen species represent a basic unifying mechanism behind atherosclerosis development under different path- ological conditions and creates a major rationale for the use of antioxidants in the prevention and treatment of cardiovascular disease **(Lo!nn et al. 2012).** There is a growing body of both epidemiological and clinical evidence that cocoa flavanols (catechins and epicatechins) and cocoa alkaloids (theobromine and the- ophylline) can be used effectively for the prevention and treatment of cardiovascular disease **(Flammer et al. 2012; Cicero and Borghi 2013).** These claims have significant support from multiple in vivo and in vitro studies describing the antioxidant effect of cocoa flavanols and alkaloids resulting in modulation of nitric oxide production and cyclic adenosine monophosphate (cAMP) turnover **(Fern_andez Vallinas et al. 2013).**

Flavonoids are a group of polyphenolic compounds that occur widely in fruit and vegetables,. Certain cocoas and chocolates can also be rich sources of flavonoids, especially the sub- class of oligomeric flavonoids known as procyanidins. Indeed, the antioxidant activity of cocoa and chocolate was shown to be correlated with its catechin and procyanidin contents **(Bearden et al., 2000).**

Cocoa and its products, such as cocoa-rich chocolate, have been known for their good taste. Cocoa products contain more polyphenols than teas. A prospective study, involving 470 elderly men, highlights the protective effects of cocoa intake in decreasing blood pressure and reducing cardiovascular disease and all-cause mortality **(Buijsse et al., 2006)**.

Chocolate and cocoa are produced from cacao beans, the seed of Theobroma cacao, and are known to contain fats (thedry weight of whole cacao beans is composed of 50–57% lipid, often called cocoa butter **(Hannum and Erdman, 2000).** This cocoa butter, predominantly found in dark chocolate, is composed on average of 33% oleic acid, 25% palmitic acid, and 33% of stearic acid.,the latter two being saturated fats. Cocoa products are also very rich in plant phytochemicals, especially flavonoids, which are now objects of increased scientific attention due to their potential health benefits **(Wang-Polagruto et al., 2006; Almoosawi et al., 2010).**

Cocoa is one of the richest flavonoid-containing foods available. Over 10 percent of the weight of cocoa powder, which is used to make beverages, is flavonoids. Chocolate also contains a small amount of theobromine (1.2%). Contrary to common belief, chocolate is not a rich source of

caffeine **(Hammerstone et al., 2000).**

A number of observational and clinical studies indicated that cocoa or cocoa-containing products may reduce cardiovascular disease (CVD) risk **(Buitrago-Lopea et al. 2011)**. and improve CVD-related risk factors such as blood pressure (2), LDL oxidation, inflammatory status (Ferna´ndez-Murga et al., 2011), and the blood lipid profile **(Tokede et al., 2011).**

Previous studies have suggested that dark chocolate consumption reduces blood pressure, improves insulin sensitivity (**Grassi et al., 2008**), improves vascular endothelial function and reverses vascular dysfunction **(Grassi et al., 2005b; Wang-Polagruto et al., 2006)**, reduces insulin resistance as evidenced by significantly lower HOMA-IR (homeostasis model assessment of insulin resistance) (**Grassi et al., 2005a**) measurements, and increases serum total antioxidant capacity (**Wan et al., 2001**).

Dark chocolate is a food consumed frequently and widely all over the world. It is therefore relevant to understand its net benefits on health in order to help the public make informed choices.

The aim of this study was to assess the effect of the administration of cocoa powder, white chocolate and dark chocolate on oxidative stress and lipid profile on hypercholesterolemic rats

2. Materials and Methods

Cocoa, white chocolate and dark chocolate were purchased from local market, Cairo, Egypt, and the chemical composition of Cocoa, white chocolate and dark chocolate were analyzed

All chemicals including cholesterol and Kits were fine grade chemicals purchased from local distributer (Sigma chemical) Cairo, Egypt.

Animals:

Forty-eight male Sprague-Dawley rats weighing 144.73 ± 8.29 were obtained from the Institute of Ophthalmology (Cairo, Egypt). The rats were allowed to acclimatize for one week, during which they were fed a control basal diet prepared in accordance with AIN-93 formulation (Reeves *et al*., 1993) and water *ad libitum*. The animals were housed in stainless steel cages at a temperature and light-controlled room.

Experimental design:

The rats were divided into two experiments:

Experiment "1" thirty-two rats were used and divided into 4 groups of 8 rats each

Group 1: (control): Rats were received standard basal diet according to AIN-93 formulation (Reevees*et al.,* 1993). (BD).

Group 2: Rats were received high fat high cholesterol diet (HFC) containing 15% fat + 1% cholesterol.

Group 3: Rats were received high fat high cholesterol diet (HFC) +5 % cocoa powder.

Group 4: Rats were received high fat high cholesterol diet (HFC) +10 % cocoa powder.

Experiment "2"Thirty-two rats were used and divided into 4 groups of 8 rats each

Group 1: (control): Rats were received standard basal diet according to AIN-93 formulation (Reevees*et al.,* 1993). (BD).

Group 2: Rats were received high fat high cholesterol diet (HFC) containing 15% fat +1% cholesterol.

Group 3: Rats were received high fat high cholesterol diet (HFC) + 2 % white chocolate (WC).

Group 4: Rats were received high fat high cholesterol diet (HFC) + 2 % dark chocolate(DC).

** Group 1 & 2 are shared in the two experiments.

Sample collection:

On completion of the experiment, all rats were fasted overnight before euthanasia. The abdominal cavity was opened and blood was withdrawn from hepatic portal vein into tubes to separate serum by centrifugation and kept at -20^{o} C for biochemical analysis.

Livers, kidney, heart and spleen were dissected out rinsed in cold isotonic saline solution, dried by blotting between filter papers and weighed.

Biochemical analysis:

Serum total cholesterol was assayed by the method of **Richmond, (1973)**, serum triacylglycerol according to **Fossati and Prencipe, (1982)**, serum HDL by the method of **Steele et al., (1976** while serum low-density lipoprotein-cholesterol (LDL-C) fraction and atherogenic index (AI) were determined according to the Friedewald equations (**Friedewald et al., 1972):**

LDL-C = Total cholesterol –(triacylglycerol/5 +HDL-C).

AI = (TC – HDL-C) / HDL-C.

Serum very low-density lipoprptein cholesterol (VLDL-C) concentration was calculated according to **Friedewald et al., (1972)** by the following equation:

Serum VLDL-C (mg/dl) = Triacylglycerols/5.

Serum total protein and albumin were determined according to the methods described by **Weichselbaum (1946)** and **Doumaset al. (1971),** respectively. Serum uric acid was determined by the method described by **Fossatiet al. (1980)**.Erythrocyte superoxide dismutase activity was determined in accordance with the method described by **Sun et al. (1988).**

Reduced glutathione (GSH) was measured in blood according to the method of **Beutler et al. (1963).**

Serum MDA was measured as an indication of lipid peroxidation using the colorimetric method described by **Draper and Hadly, (1990).** Gamma glutamyltransferase (GGT) activity was determined in

serum according to the method described by **Rosalki (1975).**

Calculation of organ: Relative weight was calculated according to the following equation:
Organ relative weight = (organ weight/ animal final body weight) x 100

Statistical analysis:

Statistical analyses were performed by using the SPSS software (version 16; SPSS Inc., Chicago, IL, USA). The results were expressed as means ± standard deviation (SD). Differences between treatment groups were analyzed by one-way analysis of variance (ANOVA) with post hoc analysis using Bonferroni multiple test. Differences were considered significant when $P < 0.05$.

3. Results:

Chemical composition of cocoa, white chocolate and dark chocolate:

Data in table (1) show that white chocolate (WC) containing more fat and more saturated fat followed by dark chocolate (DC) and then cocoa powder (CP), white chocolate also containing 20 mg cholesterol/100g while DC and CP are cholesterol free. This table also showed that cocoa powder is the richest source of fiber while white chocolate containing no fiber. DC has more carbohydrate followed by WC, then CP. On the other hand CP has more protein followed by WC, then DC.

Table 1 –Analysis ofchemical composition of cocoa, whitechocolate and dark chocolate (per 100g)

Type of chocolate	Protein (g)	Fat (g)	Saturated fat (g)	Mono-unsaturated fat (g)	Poly-unsaturated fats (g)	Carbohydrate (g)	Dietary fiber (g)	Cholesterol (mg)
Dark	5.1	28.5	17.1	9.2	1.0	62.6	1.2	0
White	7.1	33.2	20.9	9.7	1.1	54.6	0.0	20
Cocoa powder	19.1	14.3	8.6	4.7	0.4	24.3	28.0	.0

Effect of Cocoa Powder, white or dark chocolate on body weight gain and relative weight of organs:

Final body weight was significantly increased ($p<0.05$) in HFC group as compared to normal control basal diet group (BD). The addition of cocoa powder, white chocolate or dark chocolate caused non-significant decrease in final body weight in comparison with their corresponding HFC group (Tables 2 & 3).

Concerning the relative weight of liver, the results showed high significant increase ($P< 0.05$) of relative weight of liver in rats fed on HFC diets as compared with the control group (BD). The addition of cocoa powder, white chocolate or dark chocolate caused significant decrease in comparison with their corresponding HFC group. On the other hand there was no significant difference in relative weight of kidney, spleen and heart between the different treatment groups (Tables 2 & 3).

Table (2): Effect of Cocoa Powder on body weight gain and relative weight of organs in hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	Initial body weight gain (g)	Final body weight gain (g)	Relative weight of liver	Relative weight of kidney	Relative weight of spleen	Relative weight of heart
Group 1 Normal control (BD)	144.13 ± 9.5[a]	166.25 ±11.04	2.54 ± 0.012 [a]	0.56± 0.032 [a]	0.378± 0.014 [a]	0.26± 0.017 [a]
Group 2 High cholesterol diet (HFC)	145.12 ± 9.2 [a]	183.88 ±6.64 [a]	2.88 ± 0.089	0.57 ± 0.046 [a]	0.387 ± 0.021 [a]	0.25 ± 0.032 [a]
Group 3 (5% Cocoa Powder)	145.88 ± 8.77 [a]	181.25±9.91 [a]	2.53 ± 0.013 [a]	0.56 ± 0.053 [a]	0.376 ± 0.011 [a]	0.25 ± 0.012 [a]
Group 4 (10% Cocoa Powder)	144.25 ± 10.07 [a]	177.88±6.08 [a]	2.52 ± 0.073 [a]	0.56 ± 0.057 [a]	0.387 ± 0.039 [a]	0.24 ± 0.013 [a]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference ($P< 0.05$).

Table (3): Effect of white and dark Chocolate on body weight gain and relative weight of organs in hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	Initial body weight gain (g)	Final body weight gain (g)	Relative weight of liver	Relative weight of kidney	Relative weight of spleen	Relative weight of heart
Group 1 Normal control (BD)	144.13 ± 9.5 [a]	166.25 ±11.04	2.54 ± 0.012 [a]	0.56 ± 0.032 [a]	0.378 ± 0.014 [a]	0.26 ± 0.017 [a]
Group 2 High cholesterol diet (HFC)	145.12 ± 9.2 [a]	183.88 ±6.64 [a]	2.88 ± 0.089	0.57 ± 0.046 [a]	0.387 ± 0.021 [a]	0.25 ± 0.032 [a]
Group 3 (5% Cocoa Powder)	143.38 ± 6.72 [a]	183.13± 7.02 [a]	2.54 ± 0.056 [a]	0.58 ± 0.022 [a]	0.392 ± 0.034 [a]	0.27 ± 0.043 [a]
Group 4 (10% Cocoa Powder)	145.63 ± 7.54 [a]	180.63 ± 12.28 [a]	2.53 ± 0.043 [a]	0.55 ± 0.058 [a]	0.386 ± 0.037 [a]	0.26 ± 0.029 [a]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference ($P< 0.05$).

Table (4): Effect of Cocoa Powder on serum total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol VLDL-C and high density lipoprotein cholesterol (HDL-C), in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	TC (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	AI
Group 1 Normal control(BD)	105.2513±2.29	98.13±1.47	42.50±1.88	43.13±1.13	19.63±0.46	1.44±0.05
Group 2 High cholesterol diet(HFC)	176.75 ± 3.49	156.38±3.66	124.70±3.50	30.75±1.04	31.28±0.73	5.08±0.20
Group 3 (5% Cocoa Powder)	17175±2.02	128.68±1.79[a]	162.00±3.25[a]	54.53±1.90	41.75±2.12[ab]	32.40±0.65a
Group 4 (10% Cocoa Powder)	134.50±2.73	140.13±1.88[b]	173.13±5.25[b]	67.50±1.68[a]	38.00±2.14[bc]	34.63±1.05[b]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference ($P< 0.05$).

Table (5): Effect of white and dark Chocolate on serum total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol VLDL-C and high density lipoprotein cholesterol (HDL-C), in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	TC (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	AI
Group 1 Normal control(BD)	105.2513±2.29	98.13±1.47	42.50±1.88	43.13±1.13	19.63±0.46	1.44±0.05
Group 2 High cholesterol diet(HFC)	176.75 ± 3.49	156.38±3.66	124.70±3.50	30.75±1.04	31.28±0.73	5.08±0.20
Group 3 2% White Chocolate(WC)	129.25±3.49	126.25±3.05	66.0±1.39	38.0±1.41	25.25±0.77	2.4±0.04
Group 4 2% Dark Chocolate(DC)	115.25±2.82	10838±3.58	59.33±1.35	39.25±1.16	21.68±0.71	1.90±0.02

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference ($P< 0.05$).

Effect of Cocoa Powder, white or dark chocolate on serum lipid profile:

Results in tables (4&5) illustrate that HFC diet caused a significant increase (P<0.05*)* of serum total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol VLDL-C and a significant decrease (p<0.05) in serum high density lipoprotein cholesterol (HDL-C), in comparison with control basal diet group (BD). Cocoa powder (CP), white chocolate (WC) and dark chocolate (DC) treatment caused high significant decreases in serum TC, TAG, (LDL-C), VLDL-C and a significant increase (p<0.05) in serum (HDL-C). The lowest decrease of serum lipid profile (serum TC, TAG, (LDL-C), VLDL-C) and the highest increase in serum (HDL-C) was recorded in rats fed on 10% cocoa powder and 2% dark chocolate for the first and second experiment respectively.

Effect of Cocoa Powder, white or dark chocolate on serum total protein, Albumin and uric acid:

Concerning serum total protein, Albumin and uric acid, the results showed high significant increase (P< 0.05) in serum uric acid and a significant decrease in serum total protein, Albumin in rats fed on HFC diets as compared with the control group (BD). The addition of cocoa powder, white chocolate or dark chocolate caused non-significant difference in comparison with their corresponding HFC group.

Table (6): Effect of Cocoa Powder on serum total protein (TP), Albumin and serum uric acid n Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	Total protein (g/dl)	Albumin (g/dl)	Uric acid (mg/dl)
Group 1 Normal control(BD)	7.15 ± 0.49	2.81 ± 0.33 [a]	2.05 ± 0.23
Group 2 High cholesterol diet(HFC)	6.16 ± 0.27[a]	2.21 ± 0.22 [b]	2.64 ± 0.23 [a]
Group 3 (5% Cocoa Powder)	6.21 ± 0.53 [a]	2.38 ± 0.29 [b]	2.58 ± 0.19 [a]
Group 4 (10% Cocoa Powder)	6.30 ± 0.20 [a]	2.51 ± 0.24[ab]	2.42 ± 0.33 [a]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (*P*< 0.05).

Table (7): Effect of white and dark Chocolate on serum total protein (TP), Albumin and serum uric acid, in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	Total protein (g/dl)	Albumin (g/dl)	Uric acid (mg/dl)
Group 1 Normal control (BD)	7.15 ± 0.49	2.81 ± 0.33	2.05 ± 0.23
Group 2 High cholesterol diet (HFC)	6.16 ± 0.27 [a]	2.21 ± 0.22 [a]	2.64 ± 0.23 [a]
Group 3 2% White Chocolate (WC)	6.21 ± 0.39 [a]	2.39 ± 0.20 [a]	2.55 ± 0.19 [a]
Group 4 2% Dark Chocolate (DC)	6.36 ± 0.35 [a]	2.25 ± 0.26 [a]	2.58 ± 0.16 [a]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (*P*< 0.05).

Table (8): Effect of cocoa powder on serum MDA, SOD, and GPX in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	**Serum MDA (nmol\L)**	**Erythrocyte SOD** (U/ml)	**Blood GSH** (mg/dl)	**Serum GGT activity** (U/L)
Group 1 Normal control(BD)	1.85±0.07	277.5 ±3.66	28.74±0.89	13.39 ±0.38 [a]
Group 2 High cholesterol diet(HFC)	3.87±0.06	190.6 ± 3.58	18.79 ±0.63	12.15 ±0.58
Group 3 (5% Cocoa Powder)	2.30±0.07	254.6 ± 3.78	23.57 ± 0.84[a]	13.69 ±0.91 [a]
Group 4 (10% Cocoa Powder))	2.39±0.03	261.3 ±2.92	24.7 ± 0.92 [a]	13.87 ± 0.77 [a]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference (*P*< 0.05).

Table (9): Effect of white and dark Chocolate on serum MDA, SOD, and GPX in Hypercholesterolemic rats (Mean ±SD).

Parameters / Groups	Serum MDA (nmol\L)	Erythrocyte SOD (U/ml)	Blood GSH (mg/dl)	Serum GGT activity (U/L)
Group 1 Normal control(BD)	1.85±0.07	277.5 ± 3.66	28.74±0.89	13.39 ±0.38 [a]
Group 2 High cholesterol diet(HFC)	3.87±0.06	190.6 ± 3.58	18.79 ±0.63 [a]	12.15 ±0.58
Group 3 White Chocolate (WC)	3.27 ± 0.26	198.63 ± 6.14	18.92 ± 0.56 [a]	13.63 ±0.23 [a]
Group 4 Dark Chocolate (DC)	2.28 ±0.041	255.25 ± 4.30	22.88 ± 1.25	13.55 ±0.85 [a]

Values are expressed as means ± standard deviation (n = 8). Means with similar superscript (a, b c, d) letters in columns indicate non-significant difference ($P< 0.05$).

Effect of cocoa powder, white or dark chocolate onoxidative status:

Tables (8&9) show that HFC diet caused a significant increase (P<0.05) in serum MDA, and a significant decrease in erythrocyte SOD, Blood GSH and serum GGT activity as compared with the control group (BD). Administration cocoa powder, white chocolate or dark chocolate caused significant (P<0.05) decrease in the lipid peroxidation and a significant increase (p<0.05) in erythrocyte SOD, blood GSH and serum GGT activity as compared with the corresponding HFC group, with the lowest serum MDA and the highest erythrocyte SOD, blood GSH and serum GGT activity for 10 % cocoa powder group for the first experiment and 2% dark chocolate for the second experiment.

4. Discussion:

This study was performed in order to determine if 4 weeks consumption of cocoa powder, white chocolate and dark chocolate produced a different hypolipidemic and antioxidant effect in hypercholesterolemic rats.

Data in table (1) show that white chocolate (WC) containing more fat and more saturated fat followed by dark chocolate (DC) and then cocoa powder (CP), white chocolate also containing 20 mg cholesterol/100g while DC and CP are cholesterol free. This table also showed that cocoa powder is the richest source of fiber while white chocolate containing no fiber. DC has more carbohydrate followed by WC, then CP. On the other hand CP has more protein followed by WC, then DC.

Final body weight was significantly increased (p< 0.05) in HFC group as compared to normal control basal diet group (BD). The addition of cocoa powder, white chocolate or dark chocolate caused non-significant decrease in final body weight in comparison with their corresponding HFC group (Tables 2 & 3).

Concerning the relative weight of liver, the results showed high significant increase (P< 0.05) of relative weight of liver in rats fed on HFC diets as compared with the control group (BD). The addition of cocoa powder, white chocolate or dark chocolate caused significant decrease in comparison with their corresponding HFC group.

As shown in Tables 4 and 5 High fat high cholesterol (HFC) fed rats showed a significant increase in serum total cholesterol, triacylglycerol, LDL-C, v-LDL-C and atherogenic index as compared to control group (BD). On the other hand, High cholesterol fed diet rats showed a significant decrease in high-density lipoproteins (HDL).

In line with the results of the present study Consuming high fat high cholesterol diet (HFC) increases the risk of cardiovascular diseases causing hyperlipidemia and Arteriosclerotic vascular disease (ASVD) in addition to augmenting LDL-cholesterol levels over time (**Onody et al., 2003**).

The importance of plasma lipids in cardiovascular disease has been very well documented by human and animal studies. A 1% reduction in LDL cholesterol can reduce CAD risk by ’2%. Each milligram (per deciliter) reduction of LDL cholesterol can reduce CAD risk by 1% (**Jia et al., 2010**)

In this study, consumption of cocoa powder and chocolate increased significantly the concentration of HDL cholesterol and decreased significantly serum cholesterol and triglycerides concentration after 4 weeks of consumption of cocoa powder and dark chocolate. Moreover consumption of cocoa powder and chocolate decrease significantly the concentration of LDL cholesterol.

The largest decrease of serum lipid profile (serum TC, TAG, (LDL-C), VLDL-C) and the highest increase in serum (HDL-C) was recorded in rats fed on 10% cocoa powder and 2% dark chocolate for the first and second experiment respectively.

In a general sense, the results of the present study are in agreement with those of **Tokede et al., 2011** who showed a statistically significant reduction in LDL and TC after intervention with dark chocolate/cocoa products. There was also a non-significant reduction in serum TG and HDL cholesterol with ingestion of dark chocolate/ cocoa products when compared with placebo. The dark chocolate/cocoa effect seemed stronger in subjects

with higher risk of cardiovascular disease and in studies with a relatively shorter duration.

The observed reduction in serum lipids in our study 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. The degree to which LDL and TC levels were reduced in this analysis reflects some measure of potency of the cocoa regimen (**Matsui et al., 2005**)..

Our results are consistent with a similar review **Jia et al.(2010)** conducted on eight studies, which reported that intervention with dark chocolate/cocoa products led to LDL reduction by 5.87 mg/dl and TC reduction by 5.82 mg/dl compared with placebo.

On the other hand Paula **Nogueira et al. (2012)** showed that, the lipid profile had no significant modifications with dark chocolate or cocoa consumption. However, some studies observed significant reductions on total and/or LDL cholesterol after the intake of dark chocolate or cocoa (**Jia et al., 2010**). **Mursu et al. (2004)** found that the ingestion of 75 g/day of dark chocolate for 15 days increased HDL cholesterol. The changes seen in lipid profile in the studies cited above were highly dependent on the dose of cocoa consumption and health status.

The content of lipids in chocolate is high. As seen in Table 1. Despite its high fat content, cocoa itself does not seen to exert untoward effects on serum lipids (and in some studies has beneficial effects), because cocoa butter is composed on average of 33% oleic acid, 25% palmitic acid, and 33% of stearic acid. Oleic acid is a monounsaturated fat that lowers LDL cholesterol (**Cleeman et al., 2001**) and although palmitic and stearic acids are saturated fats, stearic acid in comparison with other saturated fatty acids lowers LDL cholesterol (**Mensink et al., 2003**).

One clinical trial indicated that regular ingestion of dark chocolate may have no adverse effects on serum lipid profile (**Crews et al., 2008**), whereas others have suggested that intake of dark chocolate reduced serum LDL cholesterol and tri-glyceride (TG) levels (**Engler et al., 2004; Grassi et al., 2005b**), and increased serum high-density lipoprotein (HDL) cholesterol measurements (**Mursu et al., 2004**). An earlier meta-analysis of eight randomized trials involving 215 subjects reported that an intervention with dark chocolate was associated with a significant reduction in serum LDL in subjects with cardiovascular disease risk factors (**Jia et al., 2010**) compared with placebo. However, that meta-analysis did not assess the effect of dark chocolate or cocoa on serum TG concentrations.

Jia et al. (2010) showed that short-term supplementation with cocoa products was associated with a decrease in LDL cholesterol, but had no significant effect on TC and HDL cholesterol compared with controls.

Cocoa consumption significantly decreased both LDL cholesterol and TC in the low-dose cocoa group and in participants with cardiovascular risks, whereas it had no effect on blood lipid if the cocoa dose was middle to high or in healthy people. No heterogeneity was observed in 3 of the different cocoa dose subgroups and in the cardiovascular risk subgroup. This conclusion may influence the eating habits of many people who are hesitant to eat chocolate or are addicted to chocolate. In other words, it appears to support the idea that it is good to eat moderate amounts of cocoa or dark chocolate, which may potentially benefit our health, and that cocoa products might not be “forbidden fruit” to subjects with cardiovascular risks (**Jia et al., 2010**).

In fact consumption of cocoa and dark chocolate have been demonstrated to increase the concentration of HDL cho- lesterol (**Rein et al., 2000**).

Moderate cocoa consumption may make blood cholesterol move in a healthy direction, whereas higher cocoa consumption may not affect lipid profile. Polyphenols have been shown to inhibit cholesterol absorption and biosynthesis and to promote the expression of LDL cholesterol receptors (**Jia et al., 2010**).

Cocoa butter also contains ’33% monounsaturated oleic acid, which has been shown to favor an ideal lipid profile (**Corti ET AL., 2009**). However, a high dose of polyphenols has been shown to exert cytotoxic effects on liver cells, a major metabolic organ in our body (**Schmidt et al., 2005**). The adverse effect is mainly due to (2)-epigallocatechin-3-gallate, a component of polyphenols, which exists in cocoa as well, acts as a pro-oxidant, and is cytotoxic in hepatoma cells (**Waltner-Law et al., 2002**). Therefore, higher polyphenol supplementation may counteract its beneficial biological effects on lipid metabolism. Moreover, an animal study shows that a low amount of cocoa supplementation can significantly reduce plaque, but a high amount of cocoa supplementation does not (**Vinson et al., 2006**).

It is well acknowledged that patients with cardiovascular disease or cardiovascular-related diseases such as hypertension and diabetes commonly have dyslipidemia or lipid metabolism dysfunction (**Nathan et al., 1993**).

Both human and animal studies have indicated that cocoa reduced blood cholesterol more significantly in hypercholesterolemic subjects or in animals fed a high-fat diet (**Lecumberri et al., 2007**).

A newly released study showed that polyphenols specifically targeted the pathogenesis of hyperlipidemia in diabetes to lower blood cholesterol **(Zang and Maitland-Toolan, 2006).**

Moreover, in general, cardiovascular disease or cardiovascular-related diseases share some similar pathological mechanisms such as inflammation, insulin resistance, lipid metabolism dysfunction, and oxidative stress. Previous studies indicated that cocoa could improve insulin sensitivity and antagonize inflammatory activity and oxidative stress, which are helpful in balancing lipid metabolism (**Corti et al., 2009**). Therefore, cocoa consumption might significantly improve lipid profiles in subjects with cardiovascular-related disease.

On contrast to the results of the present study, a recent meta- analysis that shows that cocoa supplementation has no effect on LDL-cholesterol and HDL-cholesterol concentrations (**Hooper et al., 2008**).

A previous study suggested that short-term of cocoa consumption reduced blood cholesterol, and this effect was more evident in studies with low- dose cocoa supplementation and in subjects with cardiovascular risks (**Jia et al., 2010**)

Several clinical studies showed that cocoa increases HDL-cholesterol concentrations (**Khan et al., 2012**), although other studies did not confirm such a beneficial effect. Low serum HDL cholesterol (,1mmol/L) is considered an independent and inverse CVD risk factor, and beyond lowering LDL cholesterol, increasing HDL cholesterol has been suggested as a secondary lipid target for reducing CVD risk (**Muniyappa et al., 2008; Wan et al., 2001**). The cardioprotective effects of cocoa are commonly attributed to cocoa flavonoids, which have been reported to influence various CVD risk factors through multiple mechanistic pathways (**Galleano et al., 2009; Huxley et al., 2003**). However, mechanistic evidence for cocoa flavonoids to affect blood lipids is lacking (**Galleano et al., 2009**). In particular, it is not clear whether flavonoids or possibly another bioactive component of cocoa (ie, theobromine) are responsible for the reported increase in serum or plasma HDL-cholesterol concentrations.

It has been shown that a daily intake of 850 mg theobromine independently and significantly increases HDL-cholesterol concentrations by 0.16 mmol/L in healthy subjects. Together with the lack of a significant main effect of cocoa and interaction effect, this result suggests that theobromine is the major active compound in cocoa that is responsible for the beneficial HDL-cholesterol–increasing effect. (**Neufingerl et a., 2013).**

It is believed that even short-term consumption of a cocoa-rich product may have a measurable positive impact on a number of cardiovascular parameters including arterial vasodilation, platelet aggregation, myocardial reperfusion, and systemic blood pressure (**Haber and Gallus 2012**).

It has been shown that dark chocolate and cocoa polyphenols can normalize serum lipids by decreasing LDL levels (**Jia et al. 2010**) and increasing HDL in plasma (**Mursu et al. 2004**).

The dark chocolate formulations with effect on lipid profile may be extremely helpful for the prevention of cardiovascular disease. Besides that, dark chocolate with an enhanced bioavailability of cocoa flavanols has a great promise as functional food. Most of the cardiovascular effects of dark chocolate are conditional and develop in a dose-dependent manner (**Davison et al. 2010**).

In contrast, there was a study which showed negative association between flavonoids-rich chocolate consumption and cardiovascular diseases among coronary artery disease (CAD) subjects. There were no significant changes observed in lipid profiles (**Farouque et al., 2006**).

The cardioprotective effect of cocoa/chocolate may be explained by this food's effect on prostacyclins. Prostacyclins, as well as their stable analogs, inhibit platelet aggregation and reduce the risk for thrombosis, vasoconstriction and, importantly, the entry of low-density LDLs into the arterial wall. In addition to prostacyclin,, the old literature on chocolate suggests that this food is particularly beneficial for asthmatics **(Schramm et al., 2001**).

The mechanisms by which cocoa/chocolate could benefit cardiovascular health is by its influence on oxidative defense mechanisms.

Concerning serum total protein, Albumin and uric acid, the results showed high significant increase ($P< 0.05$) in serum uric acid and a significant decrease in serum total protein, Albumin in rats fed on HFC diets as compared with the control group (BD). The addition of cocoa powder, white chocolate or dark chocolate caused non-significant difference in comparison with their corresponding HFC group.

The results of the present study illustrated a significant increase in serum MDA and significant decrease in erythrocyte SOD, Blood GSH and serum GGT activity on hypercholesterolemic rats.

Consumption of cocoa powder or chocolate increased significantly the concentration of serum erythrocyte SOD, Blood GSH and serum GGT activity and decreased significantly serum MDA concentration after 4 weeks.

The largest increase in erythrocyte SOD, Blood GSH and serum GGT activity observed in 10% cocoa powder at the first experiment and with 2%

dark chocolate for the second one.

The largest decrease in the indices of lipid peroxidation observed in 10% cocoa powder at the first experiment and with dark chocolate for the second one.

In agreement with the results of the present study, **Rein et al., (2000)**, demonstrated that cocoa and dark chocolate have been demonstrated to increase the concentration of HDL cholesterol () and plasma antioxidant capacity, decrease the formation of lipid oxidation products (TBARS) (**Osakabe et al., 2001),** and inhibit the oxidation of LDL ex vivo (**Mathur et al., 2002**).

Cocoa and cocoa-derived products such as chocolate with 70% or more cocoa (dark chocolate) have gained attention because of evidences that they lower blood pressure and improve endothelial function (**Corti et al., 2009**). These beneficial effects have been frequently ascribed to flavonoids, a subgroup of the polyphenolic family of antioxidant chemicals, abundantly present in fruits, vegetables, red wine,teas and cocoa. Catechin and its isomer epicatechin are types of flavonoids with strong antioxidant properties. Cocoa contains high concentrations of epicatechin and has been noted to have antioxidant content that is two times higher than that of red wine and almost three times higher than that of green tea (**Khawaja et al., 2011**).

There are several plausible mechanisms by which polyphenols may improve endothelial function and lower blood pressure. In addition to their antioxidant effects which are assumed to increase the biodisponibility of nitric oxide (NO), polyphenols have been shown to increase the formation of NO by endothelial NO synthase via increased calcium level and redox-sensitive activation of the phosphoinositide 3 (PI3)-kinase/Akt pathway. Polyphenols also (1) enhance the production of endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin and (2) inhibit the synthesis of vasoconstrictors such as endothelin-1 and the angiotensin- converting enzyme (**Stoclet et al., 2004; Corti et al., 2009**).

Cocoa intake enhanced TAC in all tissues especially in thymus. Moreover, thymus SOD and catalase activities were also dose-dependently increased by cocoa. Cocoa diet enhances thymus antioxidant defenses and influences thymocyte differentiation.**(Emma et al., 2000**).

Previous studies have shown enhanced antioxidant capacity after DC consumption (**Rein et al., 2000; Serafini et al., 2003; Wang et al., 2000**).

Cocoa and chocolate are a rich source of polyphenols. In recent years cocoa, coffee and tea have been reevaluated for their antioxidant properties, and there is increasing evidence of their role in the prevention of cardiovascular pathologies. Cocoa and chocolate are rich sources of polyphenols. The flavan-3-ol monomers, cate-chin and epicatechin, and the oligomericprocyanidins are the major flavonoids in chocolate. Recently, attention has been directed to the antioxidant potential of these flavonoids in cocoa and chocolate, and their potentially protective effects against the risk of car- diovascular disease (**Wan et al., 2001**).

It has been reported that the formation of conjugated dienes is the first step in the process leading to the formation of oxidized fatty acids (**Ferretti et al., 2004**).

The effects after cocoa powder and chocolate consumption might be due to flavonoids contained in cocoa powder and dark chocolate. Moreover, the decreased lipid peroxidation could depend on fatty acids in chocolate according to previous studies which have shown a higher inhibition of lipid peroxidation by mono- unsaturated fatty acids compared to polyunsaturated ones (**Kubes et al., 1991**).

In previous studies Procyanidins extracted from cocoa exhibited endothelium-dependent relaxation (EDR) through activation of nitric oxide synthase activity in rabbit aortic rings *in vitro* **(Karim et al., 2000).** The results were reported to be due to the tetramers and higher polymers of epicatechin, and monomers, dimers, and trimers were not capable of contributing to EDR. *In vivo* studies indicated that dark chocolate, cocoa powder and cocoa liquor suppressed the development of atherosclerotic lesions and inhibited atherosclerosis (**Vinson et al., 2006**).

Cocoa powder exerted anti cancer properties in *in vivo* studies. **Amin et al. 2004**, indicated that cocoa liquor extract lower the activity of tumor marker enzymes during hepatocarcinogenesis. Cocoa powder supplementation significantly reduces the incidence of prostate carcinogenesis compared to positive.

Cocoa contains a wide range of antioxidants, which includes soluble phenolic compounds and insoluble polymeric phenolics (**Hammerstone et al., 1999**). Cocoa bean is one of best known sources of dietary polyphenols, which comprise on average 12-18% of total weight on a dry basis (**Kim et al., 1984**). Generally, cocoa contains significant amount of procyanidin monomers, namely catechin, epicatechin and dimer to tetradecamer (**Kelm et al., 2009**).

There is a link between cocoa antioxidant and health due to the significant flavonoids content. However, the presence of methylxanthines, peptides and micronutrients could enhance or reduce the observed health effects. Factors such as bioavailability, antioxidant status, and state of subjects being studied may directly or indirectly affect the health benefits of cocoa polyphenols and the other components (**Maleyki et al., 2008).**

Cocoa is reported to have high levels of antioxidant phenolics compared to tea (**Lee et al., 2003**).
Research demonstrates that flavonoids isolated from cocoa have potent antioxidant effects *in vitro.*

The flavonoids in cocoa/chocolate, which are principally catechin and epicatechin, exist in long polymers. The procyanidins contain two, three, or up to ten of the catechin or epicatechin units linked, which is fairly distinctive (**Natsume et al., 2000**).

The mechanism(s) for flavonoids' antioxidant effect such Epicatechin and other flavonoids may not only have a direct antioxidant effect, but they may also have a sparing effect on other antioxidants such as vitamins C and E. Typically, plasma levels of vitamin C decrease very quickly, vitamin E decreases slowly, and TBARS increase. However, when epicatechin is consumed at a concentration of one micromolar, there is a sparing effect on vitamins C and E (**Lotito and** Fraga**, 2000).**

Researchers found a positive correlation between intake of procyanidins and antioxidant potential. increasing the antioxidant capacity of plasma have physiological significance, inves- tigators measured plasma 2-thiobarbituric acid reactive sub- stances (TBARS), a marker of lipid oxidation. With increasing plasma levels of epicatechin, TBARS quickly decreased, inde- pendent of subjects' total lipid levels (**Rein et al., 2000).**

Conclusion

The results suggest that cocoa powder and dark chocolate had Hypolipidemicand antioxidant effect, which may be attributable to flavonoids contained in cocoa and dark chocolate.

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