



Potential Effects of White and Dark Chocolate in Hypercholesterolemic Rats

Esraa Abo Shady, Sherif Ragab, Samah Elbanna

Department of Nutrition and Food Sciences, Faculty of Home Economics,
Menoufia University, Shibin El Kom, Egypt

*Corresponding author: Esraa Abo Shady, e-mail: esraashady0@gmail.com

ABSTRACT:

This

study aimed to examine the potential impact of white and dark chocolate on the biological, biochemical, and histological factors of hypercholesterolemic rats. Thirty-six adult male albino rats were distributed into two main groups. The first main group, which was fed a basal diet, was a negative control group. The second main group was fed a high-cholesterol diet for 45 days to induce hypercholesterolemia and then divided into five subgroups; the first subgroup was fed on a basal diet as a positive control group. The other four subgroups were given dark chocolate at 5%, 10%, white chocolate at 5% and 10%, respectively, for 28 days. The results showed a significant decrease in most lipid profile indices such as (cholesterol, triglycerides, LDL), liver function, oxidative stress markers, atherogenic index (AI), coronary risk index (CRI), cardiac risk ratio (CRR), atherogenic coefficient (AC), and atherogenic fraction (AF). On the other hand, results showed a significant increase in HDL, oxidative stress markers, and hematological parameters (WBC, RBC, HGB, PLT)—biochemical results supported by the histopathological examination of the heart and liver. These findings have the potential to significantly impact our understanding of the effects of chocolate on hypercholesterolemia and recommend the moderate consumption of dark chocolate in the diet.

Keywords: hypercholesterolemia, cholesterol, dark chocolate, white chocolate

Cite as: Abo Shady et al., 2025, Potential Effects of White and Dark Chocolate in Hypercholesterolemic Rats. 35 (2), 151-172.

DOI: 10.21608/mkas.2025.345883.1354

1. INTRODUCTION

Several diseases in humans may have hypercholesterolemia as a precursor (1). Hypercholesterolemia is one of the most severe health problems many people may face. It causing complications such as atherosclerosis or blood clots (2). Hypercholesterolemia is a metabolic disease

that can lead to increased levels of plasma low-density lipoprotein (LDL) cholesterol (3, 4). Lipoprotein disorders, linked to atherogenesis and atherosclerotic cardiovascular disease, can be reduced by lowering cholesterol and statin drugs. High triglyceride levels increase acute pancreatitis risk. However, the effectiveness of lipid-reducing drugs remains a concern,

necessitating timely evaluation and treatment (5,6, and 7).

Cocoa and dark chocolate contain flavonoids, known as procyanidins, which reduce LDL cholesterol receptor expression and cholesterol absorption, resulting in a reduction in LDL and total cholesterol levels (8). (9) discovered that flavonoids in cocoa notably lower total cholesterol levels in hypercholesterolemic rats' serum (10). The noticed decrease in cholesterol levels might be related to flavonoids contained in cocoa and dark chocolate, which are believed to inhibit cholesterol absorption and LDL cholesterol receptor expression (8).

White chocolate has a fewer antioxidant activity and polyphenol concentration than dark and milk chocolate (11). (12) found that the fatty acid retrieved from thoracic lymph and the metabolism of cholesterol were reduced in rats fed on cocoa butter compared to animals given corn oil. Thus, cocoa butter has limited bioavailability (13). Cocoa butter has been proven in experiments on rats to decrease cholesterol and triglyceride levels (14,15). So, the purpose of this study was to look into the effects of dark chocolate and white chocolate on hypercholesterolemic rats.

2. MATERIALS AND METHODS:

2.1 Materials

The study included 36 adult male albino rats (Sprague Dawley Strain), aged 10 weeks and weighing 180 ± 10 g were obtained from the Medical Insects Research Institute located in Dokki, Cairo, Egypt. Menoufia University's Institutional Animal Care and Use Committee (IACUC) granted ethical approval for this investigation (Reg. No., MUFHE /S/ NFS / 31/24).

Pure white crystalline cholesterol powder and saline solutions were purchased from TECHNOGEN Chem. Co., Egypt.

Cocoa powder, virgin coconut oil, cocoa butter, honey, and stevia were obtained from a local herbiest in Menouf, Menoufia, Egypt. Milk

powder, vanilla and sea salt were obtained from a local market in Menouf, Menoufia, Egypt.

Dark chocolate was prepared manually according to (113). It consists of 118 g cocoa powder, 105 g coconut oil, 85 g honey, 5 g pure vanilla extract, and a pinch of sea salt.

White chocolate was prepared manually according to (114). It consists of 80 g cocoa butter, 60 g milk powder, and 30 g stevia.

Hypercholesterolemia was induced in rats by feeding high-cholesterol diet [4% cholesterol (w/w) and 1% cholic acid (w/w)] for 8 weeks in order to induce hypercholesterolemia according to (16).

2.2 Methods

The rats were fed a basal diet according to (17) for 7 days for adaptation, and then divided into two main groups. The first main group fed on a basal diet as a negative control group. The second main group was (hypercholesterolemic rats) divided into five subgroups, the first subgroup was fed on basal diet as a positive control group. The other four subgroups were given dark chocolate at 5%, 10%, white chocolate at 5%, and 10%, respectively, for 28 days. The experiment involved estimating body weight and feed intake of rats, observing their behavior, and slaughtering them after 28 days. At the end of experiment, blood samples were collected according to the method described by (18). At the same time, the organs: heart and liver were removed, washed in saline solution, dried by filter paper, weighed, and stored frozen in formalin solution 10% for histopathological testing both the heart and liver were stored for histopathological testing. Biological evaluation of the different diets was carried out by determination of body weight gain (BWG) %, feed intake (FI), and feed efficiency ratio (FER) according to (19).

Serum total cholesterol was determined according to (20), serum triglycerides according to (21) and (22), HDL can be determined according to (23), VLDL-c and LDL-

c were calculated according to (24), atherogenic index (A.I) and coronary risk index (CRI) were calculated according to (25). Cardiac risk ratio (CRR) was calculated according to (26). Atherogenic coefficient (AC) was calculated according to (27). Atherogenic fraction (AF) was calculated according to (28). Catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase activity (GPX) were quantified according to (29), (30) and (31), respectively. Determination of serum ALT, AST, and ALP were carried out according to (32), (33), and (34), and the hematological Parameters (WBC, RBC, HGB, PLT) were counted according to (35).

Histopathological investigations were carried out according to (36).

The data were analyzed using a completely randomized factorial design (37) when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of ($P \leq 0.05$) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.

3. RESULTS AND DISCUSSION:

The results in Table 1, it noticed a Significant increase in BWG, FI, and FER values in positive control group compared to negative control group, while the highest BWG value was recorded in group 3 (5% dark chocolate) and the lowest was found in group 4 (10% dark chocolate). The highest FI was recorded in group 4 (10% dark chocolate), while the lowest was in group 5 (5% white chocolate), with significant differences. Group 3 (5% dark chocolate) had the highest FER, while group 6 (10% white chocolate) had the lowest, with significant differences.

High BWG in group 3 (5% dark chocolate) owing to low concentration of dark chocolate, so rats didn't completely recover. On the other side, a high concentration of dark chocolate in group 4 (10% dark chocolate) made rats significant improvement, which led to a

decrease in their weight. This finding agreed with (38) who found that the treatments employed various dosages and types of cocoa, which resulted in varying amounts of sugar, calories, and fat of the investigated products, could be one reason for the non-significant improvement in anthropometric measurements, as additional calories could have impeded the weight-reduction effects.

The significant decrease in BWG in group 4 is attributed to dark chocolate, which contains fiber and monounsaturated fats, providing a feeling of fullness for a longer period. This may lead to a reduction in daily calorie intake. According to (39), dark chocolate showed a significant reduction in body weight. On the same line, the smell of chocolate has been shown to reduce appetite, which is negatively connected to ghrelin levels (40,41). Furthermore, the findings are consistent with (42), who found that feeding on a high-fat diet considerably increased body weight, whereas eating cocoa powder significantly lowered body weight and led to body loss. Consuming cocoa also has an impact on the hydrolysis of stored triacylglycerol in adipocytes into free fatty acids and glycerol, a lipolysis marker, which reduces lipid accumulation in adipocytes (43), this mechanism leading to reducing weight.

Data given in Table 2 showed the heart, lung, liver, spleen, pancreas, and kidney weight of the positive control group was higher than that of the negative control group, with no significant differences. In the treated groups, group 6 had the highest organs weight, while group 4 had the lowest, with no significant differences.

A high-fat diet significantly affects the weight of the organs in rats, as shown in Table 2 in Group 2. This leads to various pathological changes in several organs and an increase in organ weights, particularly in the liver, pancreas, spleen, and kidneys, due to fat accumulation and inflammation. This is agreed with (44), they found that high-fat diets lead to severe steatosis, with up to 95%

of hepatocytes showing fat droplet accumulation. This results in increased liver weight and dysfunction. Also, rats on high-fat diets exhibited a 14% increase in pancreas weight and a 9% increase in spleen weight

compared to controls. This suggests an adaptive response to metabolic stress. There was a 5% increase in kidney weight, indicating potential renal stress due to fat accumulation (45).

Table (1): Effects of white and dark chocolate on body weight gain, feed intake and feed efficiency ratio of hypercholesterolemic rat

	BWG (g)	FI (g)	FER
(G1) control -ve	1.3b ± 0.17	18.56b ± 1.5	0.07ab ± 0.01
(G2) control +ve	2.11a ± 0.16	24.37a ± 1.96	0.09a ± 0.005
(G3) 5% dark chocolate	2.05a ± 0.38	23.77a ± 2.14	0.09a ± 0.02
(G4) 10% dark chocolate	1.62ab ± 0.34	24.34a ± 2.23	0.07ab ± 0.02
(G5) 5% white chocolate	1.13b ± 0.27	22.17ab ± 1.39	0.05bc ± 0.01
(G6) 10% white chocolate	0.54c ± 0.18	23.63a ± 2.35	0.03c ± 0.007
LSD	0.47	3.61	0.02

Value is represented mean + SD; means in the same column subscribed with different letters indicate significant differences between these value ($P < 0.05$).

In group 4, a slight decrease in the organs' weight was observed, and this is attributed to the dark chocolate treatment. The antioxidants present in chocolate improve the health of the tissues and cells of the organs, thereby maintaining their weight. This is agreed with (46), they found in a research of the hepatosomatic index, no significant variations were identified between groups administered with varied doses of chocolate drinks, indicating that cocoa had no negative effect on liver weight in diabetic rats. Cocoa powder supplementation significantly reduced heart enlargement associated with hypercholesterolemia, with decreases in heart weight ranging from 14% to 21% in treated groups. Also, cocoa powder reduced liver enlargement, indicating that it can protect against hypercholesterolemia-induced organ weight alterations (47). While precise data on kidney, spleen, and pancreatic weights were less commonly recorded, cocoa's antioxidant characteristics imply that it may also have protective effects on these organs (48, 49). White chocolate treatment in groups 5 and 6 also led to improved organ functions and enhanced tissues, resulting in weight improvement. This is attributed to the cocoa butter, which contains antioxidants. This is agreed with (14), they found that cocoa butter supplementation has been proven to

dramatically lower cholesterol levels, with no pathological changes identified in the heart or liver tissues of rats fed cocoa butter diets. Histological studies of the kidney and spleen revealed no harmful effects, implying that cocoa butter does not harm these organs (50). Although specific statistics on pancreatic weight were not provided, the rats' general health remained stable, indicating no negative effects on pancreatic function (14).

Data given in Table 3 showed the positive control group had the highest total cholesterol serum and triglycerides value compared to negative control group with significant differences. There is a significant decrease in the treated groups compared to the positive group. The best total of serum cholesterol and triglycerides value was recorded in group 4. Feeding rats on a diet rich in fats and cholesterol leads to liver stress, altering the pathways of cholesterol and triglyceride synthesis, resulting in increased levels in the blood. The decrease in cholesterol and triglyceride levels in group 4 after the treatment period is attributed to dark chocolate treatment, which contains polyphenols and flavonoids that possess cholesterol-lowering properties by inhibiting cholesterol synthesis, an important strategy for reducing all elevated blood lipid level and this is consistent with (51) who found that

consumption of cocoa powder-derived polyphenol extract produced a hypocholesterolemic effect, and the active ingredients were shown to be oligomeric procyanidins. Additionally, based on the increased amount of fecal sterols voided in

vivo and reduced micellar solubility in in vitro conditions, the polyphenol extract from cocoa powder may contribute to the hypocholesterolemic impact by preventing bile acid cholesterol and absorption.

Table (2): Effects of white chocolate and dark chocolate on internal row organs weight of hypercholesterolemic rats

	Heart (%)	Lungs (%)	Liver (%)	Spleen (%)	Pancreas(%)	Kidney (%)
(G 1) control -ve	0.33a +0.02	0.62a +0.09	3.27a +0.29	0.33a +0.04	0.27a +0.05	0.61a +0.12
(G 2) control +ve	0.39a +0.14	0.57a +0.17	3.46a +0.31	0.30a +0.08	0.51a +0.28	0.71a +0.09
(G 3) 5%dark chocolate	0.34a +0.01	0.61a +0.02	3.44a +0.36	0.27a +0.03	0.5a + 0.09	0.69a +0.17
(G 4) 10% dark chocolate	0.33a +0.03	0.56a +0.09	3.35a +0.82	0.26a +0.04	0.46a +0.02	0.67a +0.02
(G 5) 5% white chocolate	0.34a +0.03	0.59a +0.04	3.37a +0.16	0.28a +0.12	0.49a +0.06	0.69a +0.03
(G 6) 10% white chocolate	0.35a +0.04	0.61a +0.06	3.45a +0.17	0.29a +0.05	0.51a +0.09	0.70a +0.03
LSD	0.11	0.16	0.74	0.12	0.23	0.17

Value is represented mean + SD; means in the same column subscribed with different letters indicate significant differences between these value ($P < 0.05$).

In addition, a diet rich in cocoa powder reduced endoplasmic reticulum stress in liver. Endoplasmic reticulum stress can produce hepatic steatosis and modify cholesterol and triglyceride biosynthesis pathways. (52). Flavonoids in cocoa and dark chocolate significantly lower total cholesterol levels in hypercholesterolemic rats, attributed to procyanidins in cocoa, which impede cholesterol absorption and LDL cholesterol receptor development (9, 8).

The reduction in cholesterol and triglycerides in the groups treated with white chocolate (groups 5,6) is attributed to stearic acid in cocoa butter which decreases cholesterol absorption and enhances endogenous cholesterol excretion, leading to lower serum cholesterol levels and this is consistent with (53) they reported that dietary stearic acid significantly lowers cholesterol absorption efficiency to 21% compared to 50-55% in other fatty acids, resulting in increased fecal cholesterol excretion. (54) found that the ability of cocoa butter to neutralize cholesterol is widely recognized. Additionally, 33% of cocoa butter is composed of monounsaturated oleic acid, which has been demonstrated to support healthy lipid profile (55). Also, (49) found that white chocolate

resulted in highly noticeable decreases in T.C and T.G.

Data given in Table 4 showed a significant decrease in HDL-c value in positive control group and the treated group. There is a significant increase in the treated groups compared to the positive group. The positive control group had the highest LDL-c and v-LDL value compared to negative group, there is a significant decrease in the treated groups compared to the positive group. The best values were observed in group 4.

The observed decrease in LDL and increase in HDL in the groups treated with dark chocolate especially group 4 is due to the flavonoids and procyanidins found in dark chocolate, which improve HDL's ability to remove excess cholesterol and transport LDL from the arteries to the liver for disposal. The results have agreement with (8), who discovered that cocoa and dark chocolate contain flavonoids, or procyanidins, which can reduce LDL-c and TC levels by preventing cholesterol absorption and developing LDL cholesterol receptors and in the same way, many human studies have shown that feeding foods rich in polyphenols, including cocoa powder, altered and raised HDL-C and decreased LDL-C concentrations. RCT refers to HDL's role as a cholesterol acceptor, transporting extra cholesterol from

peripheral tissues into the liver for excretion or formation. Before being excreted, it is converted into bile acids and salts (56). Reverse cholesterol transport (RCT) is a term used to describe the efflux of excess cellular cholesterol from peripheral tissues and its return to the liver for excretion in the bile and ultimately the feces (57). The neutral saturated fatty acids found in cocoa fat are

mostly palmitate and stearate, and it also has antioxidants. Cocoa fat's antioxidants can prevent free radicals from forming, which can reduce LDL cholesterol (58). According to (59), Several research studies the health benefits of dark chocolate on blood lipid levels, such as lowering LDL-C and increasing HDL-C.

Table (3): Effects of white and dark chocolate on total cholesterol and triglycerides of hypercholesterolemic rats

	T.C (mg/dl)	T.G (mg/dl)
(G1) control –ve	96.72e ± 1.52	89.41e ± 2.92
(G2) control +ve	200.33a ± 2.45	148.8a ± 2.25
(G3) 5% dark chocolate	132.31b ± 2.37	127.39b ± 3.05
(G4) 10% dark chocolate	116.24d ± 1.78	91.91e ± 2.33
(G5) 5% white chocolate	142.85b ± 1.72	122.29c ± 2.99
(G6) 10% white chocolate	128.58c ± 3.11	106.17d ± 1.87
LSD	3.96	4.71

Value is represented mean + SD; means in the same column subscribed with different letters indicate significant differences between these value ($P < 0.05$).

Although cocoa butter contains polyphenols and flavonoids fewer than cocoa, it has reduced LDL-c. This is due to stearic acid, which liver can convert stearic acid into oleic acid, a monounsaturated fat. This acid lowers levels LDL-c and increases levels of HDL-c in groups 4 and 5 that were treated with white chocolate and this agreed with (60), who found that Oleic acid in cocoa butter reduces LDL levels by decreasing the risk of coronary heart disease. The average composition of

cocoa butter is 25% palmitic acid, 33% stearic acid, and 33% oleic acid, one monounsaturated lipid that reduces LDL cholesterol is oleic acid (61) and while both stearic and palmitic acids are saturated fats, stearic acid reduces LDL cholesterol more than other saturated fatty acids (62). Also (49) found that white chocolate resulted in highly noticeable decreases in LDL and v-LDL and a noticeable increase in HDL.

Table (4): Effects of white chocolate and dark chocolate on HDL, LDL and VLDL of hypercholesterolemic rats

	HDL-c (mg-dl)	LDL-c (mg-dl)	vLDL-c (mg-dl)
(G1) control -ve	49.18e ± 1.86	29.66e ± 1.99	17.88c ± 2.36
(G2) control +ve	43.99b ± 1.73	127.18a ± 3.00	29.16a ± 2.13
(G3) 5% dark chocolate	47.75b ± 1.82	59.08c ± 2.71	25.48ab ± 1.93
(G4) 10% dark chocolate	44.63b ± 1.73	53.43d ± 1.92	18.18c ± 0.88
(G5) 5% white chocolate	46.01ab ± 1.68	72.38b ± 1.61	24.46ab ± 2.28
(G6) 10% white chocolate	47.18ab ± 2.28	59.74c ± 1.8	21.46bc ± 3.35
LSD	3.31	4.01	4.04

Value is represented mean + SD; means in the same column subscribed with different letters indicate significant differences between these value ($P < 0.05$).

Table 5 showed that AI, CRR, CRI, AC and AF values of the positive control group was highest compared to negative group with

significant differences. There is a significant decrease in the treated groups compared to

the positive group The best AI, CRR, CRI, AC and AF values was recorded in group 4. The decreased in AI, CRR, CRI, AC and AF values in group 4 due to treatment with dark chocolate led to a significant improvement in cardiovascular health and the prevention of arteriosclerosis, due to the flavonoids present in cocoa, such as epicatechin and catechin, which possess anti-inflammatory and antioxidant properties. Flavonoids in general have properties that enhance heart health and cardiac circulation, improve heart muscle function, and prevent blood clots. Consequently, a significant improvement occurred in the groups treated with dark chocolate and this is consistent with (82) and (83) they found that cocoa flavonoids have considerable antioxidant activity and reduce oxidative stress, which is a major factor to cardiovascular disease. That also have anti-inflammatory characteristics, blocking pro-inflammatory mediators and decreasing lipid peroxidation. Also, cocoa consumption improves endothelial function, encouraging vasodilation and boosting blood flow, which is important for cardiovascular health (84, 85). (86) reported that many clinical and observational research suggested that food items containing cocoa may help prevent cardiovascular disease. Consuming cocoa-rich products can positively impact cardiovascular parameters like myocardial reperfusion, platelet aggregation, arterial vasodilation, and

systemic blood pressure in the short term (87). Research conducted in vivo revealed that cocoa powder, dark chocolate, and cocoa liquor prevented atherosclerosis and reduced the growth of atherosclerotic plaques (88). Clinical studies and experimental studies have mostly examined the effects of cocoa products and cocoa polyphenols on platelet function, blood pressure, vascular inflammation, lipid profile, endothelium-dependent on vasomotor function, arterial flow-mediated dilatation (FMD), plasma antioxidant capacity, oxidative stress, and nitric oxide (NO) activity and metabolism (89, 90). So, after these effects, studied in clinical and experimental studies, are achieved, it is expected that heart health will improve.

Although cocoa butter has a lower flavonoid content than cocoa powder, it has played a role in improving heart health through biologically active flavonoid compounds. Which contribute to cardiovascular health by reducing inflammation, improving endothelial function, and enhancing blood lipid levels. This is consistent with another study reported that cocoa butter contains flavonoids, which have been proven to increase endothelium vasodilation and reduce oxidative stress (91, 85). Also, cocoa butter is high in polyphenolic chemicals, which have strong antioxidant properties and assist to neutralize free radicals and reduce oxidative stress (92).

Table (5): Effects of white chocolate and dark chocolate on AIP, CRR, CRI, AC and AF of hypercholesterolemic rats

	AI	CRR	CRI	AC	AF
(G1) control - ve	0.039a ± 5.77	1.95c ± 0.12	0.59c ± 0.07	0.95c ± 0.12	46.97c ± 6.18
(G2) control +ve	0.05a ± 0.02	4.5a ± 0.43	2.85a ± 0.38	3.5a ± 0.43	156.71a ± 17.3
(G3) 5% dark chocolate	0.043a ± 0.002	3.35b ± 0.36	1.77b ± 0.3	2.35b ± 0.36	103.19b ± 8.78
(G4) 10% dark chocolate	0.04a ± 0.003	2.73b ± 0.07	1.28b ± 0.03	1.7b ± 0.06	78.34b ± 5
(G5) 5% white chocolate	0.041a ± 0.002	2.9b ± 0.34	1.42b ± 0.3	1.9b ± 0.34	96.57b ± 13.35
(G6) 10% white chocolate	0.044a ± 0.004	2.74b ± 0.34	1.3b ± 0.31	1.74b ± 0.34	81.81b ± 2.36
LSD	0.01	0.55	0.48	0.55	19.02

Value is represented mean ±SD; means in the same column subscribed with different letters indicate significant differences between these value ($P < 0.05$).

Data given in Table 6 showed a significant increase in ALT, AST and ALP value in positive

group compared to negative group. There is a significant decrease in the treated groups

compared to the positive group. The best values were found in group 4 (rats fed on 10% dark chocolate).

Due to antioxidants present in cocoa, dark chocolate treatment has protective effects on the liver, especially in reducing serum liver enzyme levels such as ALT, AST, and ALP in group 4, which are believed to be signs of liver damage. It also reduces inflammation and oxidative stress associated with liver damage and improves the condition of damaged hepatic blood vessels. and this is consistent with (63, 64), they found that Polyphenols, which are abundant in cocoa and have strong antioxidant properties, reduce oxidative stress in the liver. Also, consuming cocoa significantly decreased blood ALT and AST levels in animal models, suggesting reduced liver damage. For example, when sick mice consumed cocoa, their ALT and AST levels decreased by 47% and 48%, respectively (65). Furthermore, cocoa has been demonstrated to reduce ALP levels, which supports its role in improving liver

function. These results suggest that cocoa may be a useful dietary supplement for people who are at risk of liver damage from health conditions like infections or alcohol consumption, possibly providing a natural substitute for traditional treatments (66, & 67). Also, the result have agreement with (68), that found information about the impact of dark chocolate consumption on the decrease in AST levels among patients with non-alcoholic fatty liver disease (NAFLD) by reducing hepatic fat accumulation, inflammation and necrosis (69).

This study showed that cocoa butter also has protective benefits on liver functions, especially concerning liver enzymes such as ALT, AST, and ALP, by including anti-inflammatory properties, modulating fat metabolism, and reducing oxidative stress. According to (70, 67), cocoa butter significantly lowered ALT and AST levels in rats fed chronic ethanol, indicating a preventive action against liver damage.

Table (6): Effects of white chocolate and dark chocolate on ALT, AST and ALP of hypercholesterolemic rats

	ALT (U/L)	AST (U/L)	ALP (U/L)
(G1) control -ve	43.57f ± 1.34	93.09f ± 2.5	133.39f ± 2.18
(G2) control +ve	140.15a ± 1.25	204.52a ± 2.14	363.37a ± 2.72
(G3) 5% dark chocolate	122.38b ± 2.19	164.17b ± 2.06	252.14c ± 1.85
(G4) 10% dark chocolate	72.06e ± 4.42	98.56e ± 1.47	214.42e ± 2.05
(G5) 5% white chocolate	86.47c ± 1.52	133.54c ± 2.06	287.6b ± 1.56
(G6) 10% white chocolate	81.08d ± 2.52	104.96d ± 1.93	239.62d ± 1.48
LSD	4.38	3.65	3.7

Value is represented mean + SD; means in the same column subscribed with different letters indicate significant differences between these value ($P < 0.05$).

Data given in Table 7 showed catalase (CAT) and glutathione peroxidase (GPX) values of the positive control group were the lowest compared to negative group. There is a significant increase in the treated groups compared to the positive group. The best CAT and GPX value recorded for group 4 (rats fed on 10% dark chocolate). The study found significant increase in malondialdehyde (MDA) value in positive group compared to negative group. There is a significant decrease in the treated groups compared to the positive group. The best value recorded for group 4.

The significant increase in CAT and GPX and the significant decrease in MDA in Group 4 are attributed to the significant biochemical effects of cocoa powder in dark chocolate, especially in modifying the levels of oxidative stress indicators such as MDA, GPX, and CAT. This is mainly because of the antioxidant properties of polyphenolic compounds in cocoa powder, which enhance the body's defenses against oxidative damage, delay cell damage, and reduce inflammation levels, thereby maintaining cardiovascular health. this is consistent with (71) they found that

dark chocolate consumption has been shown in studies to reduce MDA levels, a marker of oxidative stress, implying a cardiovascular preventive effect. Elevated MDA levels are related with a higher risk of coronary heart disease, whereas GPx is an essential antioxidant enzyme that can reduce oxidative damage (72). According to (73) In arthritic female wister rats, cocoa supplementation has been found to enhance catalase levels. (74) they found that cocoa administration may boost CAT activity by regulating NF-kB transcription factor and reducing DNA damage, while also decreasing inflammation markers like IL-6, hs-CRP, and TNF- α , potentially reducing MDA levels. This is because there was a connection between inflammatory markers and MDA, as shown in a study by (75). (76) found that the Patients

with type 2 diabetes who took cocoa supplements saw lower levels of glutathione and catalase after three months of treatment. Consuming cocoa enhances serum antioxidant capacity, which protects the endothelium from endogenous ROS and oxidative stress (77). Also, (78) found that treatment with cocoa increased the glutathione transferase and catalase level activities compared with the positive control group, while the malonaldehyde content had the opposite trend. Antioxidants in cocoa can delay or prevent cellular damage by chelating transition metal ions or quenching free radicals and reducing their ability to produce reactive oxygen species. They also demonstrate a variety of physiological features that protect against diseases such as coronary heart disease.

Table (7): Effects of white chocolate and dark chocolate on CAT, MDA and GPX of hypercholesterolemic rats

	CAT (Ng/ml)	MDA (nmol/ml)	GPX (U/ml)
(G1) control -ve	10.54a \pm 1.61	0.94c \pm 0.05	205.7a \pm 2.08
(G2) control +ve	0.86d \pm 0.003	9.64a \pm 0.67	43.97e \pm 1.03
(G3) 5% dark chocolate	2.62cd \pm 0.6	2.69b \pm 0.63	84.55d \pm 1.79
(G4) 10% dark chocolate	5.5b \pm 1.47	2.6b \pm 0.73	156.59b \pm 1.3
(G5) 5% white chocolate	4.59bc \pm 1.11	2.83b \pm 0.75	109.74c \pm 1.82
(G6) 10% white chocolate	4.25bc \pm 0.99	3.95b \pm 0.51	112.58c \pm 1.73
LSD	1.96	1.08	3.19

Value is represented mean + SD; means in the same column subscribed with different letters indicate significant differences between these value ($P < 0.05$).

Cocoa butter plays a significant role in modulating the mechanisms of CAT, MDA and GPX activities, which are important for oxidative stress management. The interaction of cocoa butter with specific phospholipids and its physicochemical properties influences these mechanisms, enhancing the antioxidant capacity of cocoa butter products. According to (79), the emulsifying qualities of cocoa butter, especially when paired with substances like PGPR, might stabilize cellular structures and possibly increase CAT activity, which is essential for the detoxification of hydrogen peroxide. Also, (80) found that the enzymatic activity of GPX, which protects against oxidative damage, can be enhanced by the

incorporation of cocoa butter. Its unique triglyceride profile may support the enzyme's function, promoting the reduction of peroxides. Cocoa butter's fatty acid composition can influence lipid peroxidation, leading to MDA production. The presence of antioxidants in cocoa butter may mitigate this process, reducing MDA levels (81). (49) found that lipid peroxidation was significantly reduced by white chocolate.

Data given in Table 8 found significant increase in RBC in positive group compared to negative group. There is a significant increase in the treated groups compared to the positive group. The best RBC value was observed in group 4. The study found significant increase

in WBC value in positive group compared to negative group. There is a significant decrease in the treated groups compared to the positive group. The best WBC value was found in group 4. The study found significant decrease in HGB value in positive group compared to negative group. There is a significant increase in the treated groups compared to the positive group. The best HGB value was observed in group 4. The PLT value of positive group recorded the lowest value when compared with negative group. There is a significant increase in the treated groups compared to the positive group. The best value of PLT recorded for group 4.

The significant increased in RBC and HGB in group 4 due to dark chocolate that contains iron, but in small amounts. However, it still improves the process of hemoglobin synthesis. Additionally, the flavonoids present in dark chocolate protect red blood cells from damage, thereby enhancing hemoglobin synthesis. According to (93) and (94), cocoa powder has a varied effect on hemoglobin levels and iron bioavailability. According to research, cocoa contains iron, however at a moderate bioavailability compared to other sources such as ferrous sulfate. According to studies using the hemoglobin regeneration efficiency method, cocoa powder has a relative biological value for iron of approximately 0.46-0.48, showing that it is a major but less efficient source of iron. Furthermore, cocoa flavonoids including epicatechin and catechin have been demonstrated to increase erythrocyte tolerance to oxidative stress, potentially lowering hemolysis and enhancing overall hemoglobin stability (95).

High white blood cell count in the affected group (group 2) can indicate a range of conditions, including inflammation, infections, injury, and immune system disorders. Therefore, due to the flavonoids and flavonoids present in dark chocolate, they inhibited white blood cells by locating the site

of inflammation or infection in the body and releasing antibodies to eliminate it therefore, white blood cell decreased in group 4. This is consistent with (96) and (97) they found that cocoa flavanols can inhibit the activation of neutrophils and monocytes, which are crucial components of the immune response. Also, in vitro studies show that cocoa procyanidins can modulate signaling pathways in polymorphonuclear cells, potentially reducing inflammation.

Treatment with dark chocolate in group 4 led to increased platelet count due to flavonoids and polyphenols, which modify platelet function, thereby enhancing their count. Moreover, it does not stop there; it also maintains their normal level in the blood, which is beneficial in preventing thrombosis. This is consistent with (98) found that cocoa flavones have been observed to suppress platelet aggregation in both healthy individuals and those with cardiovascular problems, indicating a preventive effect against thrombotic events. Also, in patients receiving dual antiplatelet medication, cocoa consumption improved clopidogrel's inhibitory action, resulting in a significant reduction in platelet reactivity (99). Cocoa polyphenols boost endothelial nitric oxide synthase activity, which is important for regulating platelet aggregation and enhancing vascular health (100). Also, (96) found in vitro and ex vivo studies have found that cocoa flavanols, notably epicatechin and catechin, significantly reduce platelet aggregation. According to (101), cocoa boosts immunity, which results in extraordinary health. Several diseases are prevented by cocoa powder, especially viral illnesses. (102) found a significant increase in red blood cells, white blood cells, and platelets in the experimental group compared to control group. It is thought that consuming a product high in cocoa, even for just a short period of time, may positively affect several cardiovascular indicators, such as platelet counts (87).

Table (8): Effects of white chocolate and dark chocolate on red blood cells, white blood cells, hemoglobin and platelets of hypercholesterolemic rats

	RBC ($10^3/\text{mm}^3$)	WBC ($10^3/\text{mm}^3$)	HGB (g/dl)	PLT ($10^3/\text{mm}^3$)
(G1) control -ve	4.57b \pm 0.59	7.33c \pm 1.08	13.69a \pm 0.86	851.67a \pm 50.08
(G2) control +ve	2.66b \pm 0.59	14.44a \pm 1.16	8.36b \pm 0.92	318.11c \pm 70.08
(G3) 5% dark chocolate	3.63ab \pm 0.69	11.52b \pm 1.08	11.23a \pm 0.98	552.89b \pm 55.12
(G4) 10% dark chocolate	4.39a \pm 0.7	8.92bc \pm 1.07	13.29a \pm 1.04	661.22b \pm 81.02
(G5) 5% white chocolate	4.16ab \pm 0.5	10.14b \pm 1.16	12.59a \pm 1.32	596.89b \pm 111.02
(G6) 10% white chocolate	4.05ab \pm 0.65	9.45bc \pm 1.34	12.45a \pm 1.04	619.56b \pm 128.78
LSD	1.11	2.05	1.85	155.65

Value is represented mean + SD; means in the same column subscribed with different letters indicate significant differences between these value ($P < 0.05$).

Histopathological examination of heart:

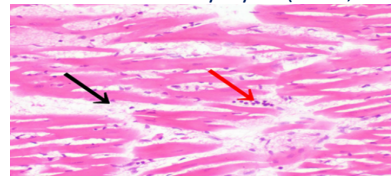
Examined heart sections of rats from group 1 (negative control group) revealed the normal histological architecture of cardiomyocytes (Photo 1). In adverse, heart of rats from group 2 (positive control group) showed severe histopathological changes manifested by severe vacuolization of the sarcoplasm of cardiac myocytes (Photo 2), intermyocardial edema and mononuclear cell infiltration (Photo 3). Meanwhile, heart of rats from group 3 (5% dark chocolate) exhibited vacuolization of the sarcoplasm of some cardiac myocytes and congestion of cardiac blood vessel (Photo 4) and slight intermyocardial edema (Photo 5). On the other hand, heart of rats from group 4 (10% dark chocolate) described no histopathological changes (Photo 6) except slight intermyocardial edema in some sections (photo 7). Furthermore, some heart of rats from group 5 (5% white chocolate) exhibited congestion of cardiac blood vessels (Photo 8), whereas other sections showed apparent normal cardiac tissue (Photo 9). Likewise, heart sections of rats from group 6 (10% white chocolate) revealed histologically normal cardiac tissue (Photo 10) except slight congestion of cardiac blood vessel (Photo 11) was seen in some sections.

In this study, severe vacuolization of the sarcoplasm of cardiac myocytes and intermyocardial edema in hypercholesterolemic rats owing to high fat diet that causes cardiovascular disorders, Increasing levels of inflammation in heart tissues and deterioration of cardiac contractility. This finding agreed with (103) they found The high-fat diet alters the amounts of SFAs and MUFAs in the membrane phospholipids, which results in varying degrees of detrimental cardiac alterations. Also, according to (104) and (105), high fat diet causes lipotoxic cardiomyopathy and cardiac hypertrophy.

Dark chocolate treatment that contains bioactive compounds such as flavonoids (catechins, epicatechins, e.g.), methylxanthines (theobromine, caffeine, e.g.), and polyphenols leads to inhibition of inflammation in heart. Additionally, dark chocolate has been shown to have anti-inflammatory and free-radical scavenging properties which leads to the improvement of heart cells and tissues, and this is consistent with another study found that cocoa prevent endothelial dysfunction and cocoa has anti-inflammatory and antioxidant properties due to its high polyphenol content which results in the enhancement of cardiac tissues and cells (106, 107, and 108).



Photo(1): Photomicrograph of heart of rat from group 1 (negative control group) showing the normal histological architecture of cardiomyocytes (H & E, X 400).



Photo(3): Photomicrograph of heart of rat from group 2 (positive control group) showing intermyocardial edema (black arrow) with mononuclear cell infiltration (red arrow) (H & E, X 400).



Photo(5): Photomicrograph of heart of rat from group 3 (5% dark chocolate) showing slight intermyocardial edema (black arrow) (H & E, X 400).

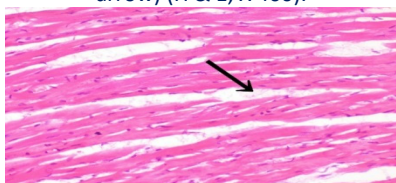


Photo (7): Photomicrograph of heart of rat from group 4 (10% dark chocolate) showing slight intermyocardial edema (black arrow) (H & E, X 400).

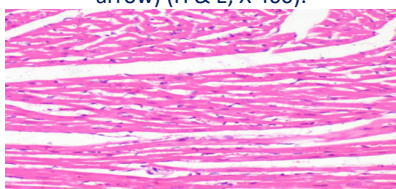


Photo (9): Photomicrograph of heart of rat from group 5 (5% white chocolate) showing apparent normal cardiac tissue (H & E, X 400).

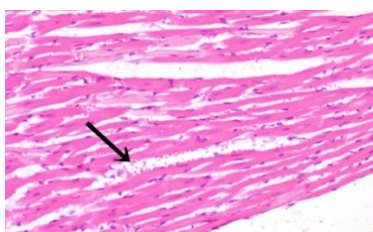
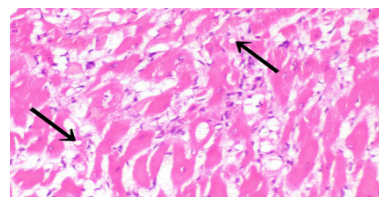
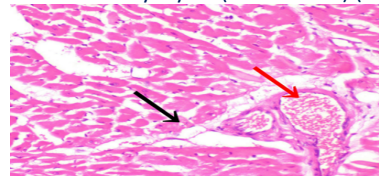


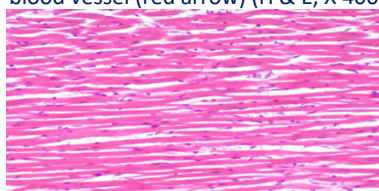
Photo (11): Photomicrograph of heart of rat from group 6 (10% white chocolate) showing slight congestion of cardiac blood vessel (black arrow) (H & E, X 400).



Photo(2): Photomicrograph of heart of rat from group 2 (positive control group) showing severe vacuolization of the sarcoplasm of cardiac myocytes (black arrow) (H & E, X 400).



Photo(4): Photomicrograph of heart of rat from group 3 (5% dark chocolate) showing vacuolization of the sarcoplasm of some cardiac myocytes (black arrow) and congestion of cardiac blood vessel (red arrow) (H & E, X 400).



Photo(6): Photomicrograph of heart of rat from group 4 (10% dark chocolate) showing no histopathological changes (black arrow) (H & E, X 400).

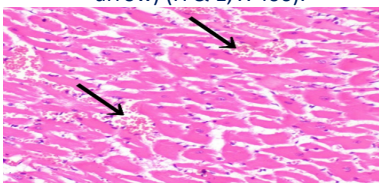


Photo (8): Photomicrograph of heart of rat from group 5 (5% white chocolate) showing congestion of cardiac blood vessels (black arrow) (H & E, X 400).

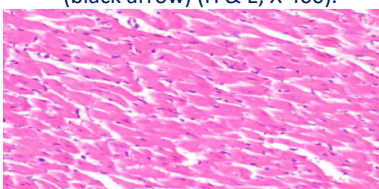


Photo (10): Photomicrograph of heart of rat from group 6 (10% white chocolate) showing histologically normal cardiac tissue (H & E, X 400).

Histopathological examination of liver:

Histopathological examination of liver sections of rats from group 1 (negative control group) revealed the normal histoarchitecture of hepatic parenchyma (Photo 1). In contrast, liver of rats from group 2 (positive control group) showed histopathological damage characterized by remarkable hepatocellular vacuolar degeneration (Photo 2) and focal hepatocellular necrosis with inflammatory cells infiltrates (Photo 3). Otherwise, liver of rats from group 3 (5% dark chocolate)

revealed regression of the induced damage; some examined sections from this group exhibited slight hydropic degeneration of some hepatocytes (Photo 4) and vacuolization of sparse hepatocytes (Photo 5), whereas other sections revealed vacuolization of centrilobular hepatocytes (Photo 6). Furthermore, hepatic tissue of rats from group 4 (10% dark chocolate) described only small vacuoles in the cytoplasm of sparse hepatocytes (Photo 7). Moreover, some hepatic sections of rats from group 5 (5% white chocolate) demonstrated small vacuoles in the cytoplasm of sparse hepatocytes (Photo 8), whereas other sections showed vacuolization of centrilobular hepatocytes (Photo 9). On the other hand, examined sections from group 6 (10% white chocolate) exhibited small vacuoles in the cytoplasm of sparse hepatocytes (photo 10) and slight activation of Kupffer cells (Photo 11). Some examined hepatic sections from group 6 (10% white chocolate) showed hepatocellular vacuolar degeneration around the portal triad (Photo 12).

Feeding rats on a high-fat diet causes liver condition deterioration, fatty liver, fibrosis, and inflammation due to the accumulation of fat in the liver and the liver's failure to process, break down, and eliminate fat properly. This is evident in the histological examination of group 2 in photos 2 and 3. This is agreed with (109) they found that high-fat diet activates hepatic stellate cells, which promote fibrosis via inflammatory pathways and cytokine signaling. High fat diet causes increased lipid buildup in hepatocytes, which results in nonalcoholic fatty liver disease (110).

The regression of the induced damage in photo 4,5,6 and 7 in groups 3 and 4 is due to

the dark chocolate treatment, which primarily benefits liver cells due to its high polyphenol content, affecting a variety of metabolic and defensive pathways. These processes include regulating lipid metabolism, enhancing the antioxidant response, and activating autophagy, all of which contribute to liver health and protect against disorders such as non-alcoholic fatty liver disease. (NAFLD). According to (111), phytochemicals found in cocoa shells activate pathways that limit lipid buildup and fatty acid production, encouraging improved lipid metabolism in hepatocytes. Also, dietary cocoa supplementation in rats boosted antioxidant enzyme activity and decreased lipid peroxides, indicating a protective effect against oxidative damage (112).

There was also a slight regression of the induced damage in groups 5 and 6 in photo 8,9,10,11 and 12 due to cocoa butter, which has protective effects on liver cells. Especially in the context of fat accumulation and inflammation, it can mitigate the harmful effects of liver damage, reducing hepatitis and fat accumulation, thereby enhancing healthy liver function and reducing oxidative stress. According to (70) in rats fed an alcoholic diet, cocoa butter reduced lipid alterations and inflammatory cell infiltration in liver tissues. It also decreased plasma levels of inflammatory markers such TNF- α and IL-1 β , indicating a decrease in liver inflammation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

No fund has been received.

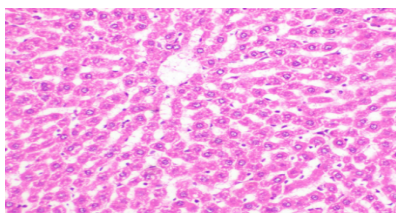


Photo (1): Photomicrograph of liver of rat from group 1 (negative control group) showing the normal histoarchitecture of hepatic parenchyma (H & E X 400).

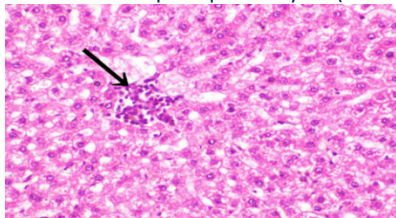


Photo (3): Photomicrograph of liver of rat from group 2 (positive control group) focal hepatocellular necrosis with inflammatory cells infiltrates (black arrow) (H & E X 400).

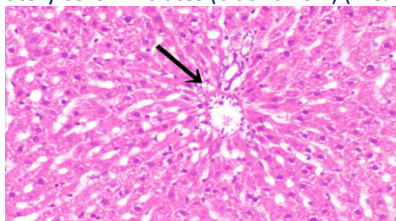


Photo (5): Photomicrograph of liver of rat from group 3 (5% dark chocolate) showing vacuolization of sparse hepatocytes (black arrow) (H & E X 400).

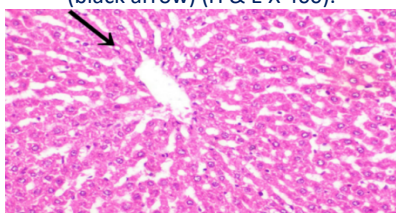


Photo (7): Photomicrograph of liver of rat from group 4 (10% dark chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

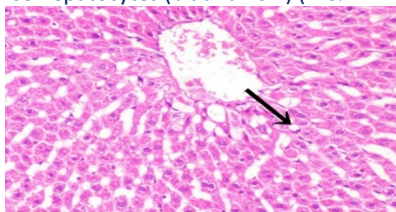


Photo (9): Photomicrograph of liver of rat from group 5 (5% white chocolate) showing vacuolization of centrilobular hepatocytes (black arrow) (H & E X 400).

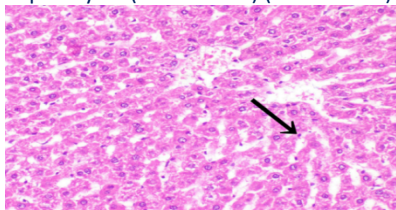


Photo (11): Photomicrograph of liver of rat from group 6 (10% white chocolate) showing slight activation of Kupffer cells (black arrow) (H & E X 400).

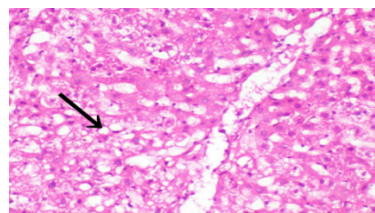


Photo (2): Photomicrograph of liver of rat from group 2 (positive control group) showing remarkable hepatocellular vacuolar degeneration (black arrow) (H & E X 400).

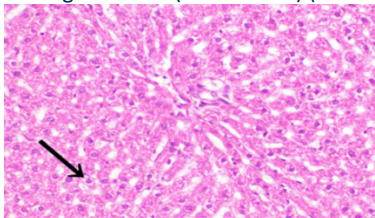


Photo (4): Photomicrograph of liver of rat from group 3 (5% dark chocolate) showing slight hydropic degeneration of some hepatocytes (black arrow) (H & E X 400).

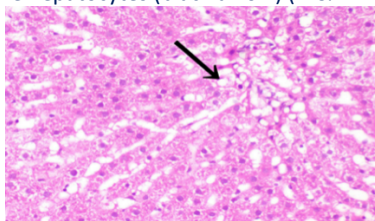


Photo (6): Photomicrograph of liver of rat from group 3 (5% dark chocolate) showing vacuolization of centrilobular hepatocytes (black arrow) (H & E X 400).

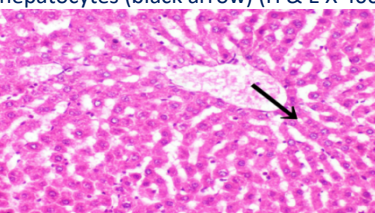


Photo (8): Photomicrograph of liver of rat from group 5 (5% white chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

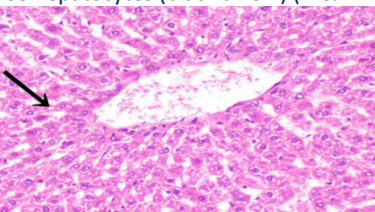


Photo (10): Photomicrograph of liver of rat from group 6 (10% white chocolate) showing small vacuoles in the cytoplasm of sparse hepatocytes (black arrow) (H & E X 400).

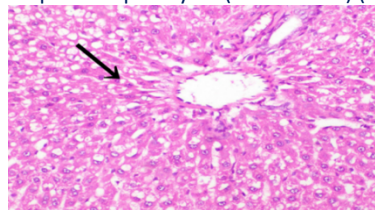


Photo (12): Photomicrograph of liver of rat from group 6 (10% white chocolate) showing hepatocellular vacuolar degeneration around the portal triad (black arrow) (H & E X 400).

4. REFERENCES

1. El-Kholie E, Ahmed A, El-Sharkawy M. Potential effects of ungerminated, and germinated quinoa seeds (*Chenopodium quinoa*, W.) on hypercholesterolemic rats. *J Home Econ-Menoufia Univ.* 2023;33(02):75-86. <https://doi:10.21608/MKAS.2023.176554.1192>
2. Sobh A, Shalan M. Studying the effect of turnip leaves and their cells on hypersensitivity in growing mice. *J Home Econ Menoufia Univ.* 1999;32(4):27-41. Available from: https://mkas.journals.ekb.eg/article_271291_683f7f84912abb9138c8e7f7f4ee824c.pdf
3. Adekiya TA, Shodehinde SA, Aruleba RT. Antihypercholesterolemic effect of unripe *Musa paradisiaca* products on hypercholesterolemia-induced rats. *J Appl Pharm Sci.* 2018;8(10):90–97. <https://DOI:10.7324/JAPS.2018.81012>
4. Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Forman JP, Missmer SA. Association between endometriosis and hypercholesterolemia or hypertension. *Epidemiol Popul.* 2017;7:59-65. <https://DOI:10.1161/HYPERTENSIONAHA.117.09056>
5. Radaelli G, Sausen G, Cesa CC, Portal VL, Pellanda LC. Secondary Dyslipidemia In Obese Children - Is There Evidence For Pharmacological Treatment? *Arq Bras Cardiol.* 2018;111(3):356–61. <https://DOI:10.5935/abc.20180155>
6. Zawacki AW, Dodge A, Woo KM, Ralphe JC, Peterson AL. In pediatric familial hypercholesterolemia, lipoprotein(a) is more predictive than LDL-C for early onset of cardiovascular disease in family members. *J Clin Lipidol.* 2018;12(6):1445–1451. <https://doi:10.1016/j.jacl.2018.07.014>
7. Wiegman A. Lipid Screening, Action, and Follow-up in Children and Adolescents. *Curr Cardiol Rep.* 2018;20(9):80. <https://doi:10.1007/s11886-018-1014-7>
8. Matsui N, Ito R, Nishimura E, Yoshikawa M, Kato M, Kamei M, et al. Ingested cocoa can prevent high-fat diet-induced obesity by regulating the expression of genes for fatty acid metabolism. *Nutr.* 2005;21(5):594601. <https://DOI:10.1016/j.nut.2004.10.008>
9. Kollar P, Kotolová H, Necas J, Karpísek M, Kris-Etherton PM, Keen CL. Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. *Curr Opin Lipidol.* 2002;13(1):41-9. <https://DOI:10.1097/00041433-200202000-00007>
10. Baba S, Natsume M, Yasuda A, Nakamura Y, Tamura T, Osakabe N, et al. Plasma LDL and HDL cholesterol and oxidized LDL concentrations are altered in normo- and hypercholesterolemic humans after intake of different levels of cocoa powder. *J Nutr.* 2007;137(6):1436–1441. <https://DOI:10.1093/jn/137.6.1436>
11. Meng CC, Jalil AM, Ismail A. Phenolic and theobromine contents of commercial dark, milk and white chocolates on the Malaysian market. *Molecules.* 2009;14(1):200–9. <https://DOI:10.3390/molecules14010200>
12. Chen IS, Subramaniam S, Vahouny GV, Cassidy MM, Ikeda I, Kritchevsky D. A comparison of the digestion and absorption of cocoa butter and palm kernel oil and their effects on cholesterol absorption in rats. *J Nutr.* 1989;119(11):1569–1573. <https://DOI:10.1093/jn/119.11.1569>
13. Weisburger JH. Chemopreventive effects of cocoa polyphenols on chronic diseases. *Exp Biol Med (Maywood).* 2001;226(10):891–7. <https://DOI:10.1177/153537020122601003>
14. Morrissey RB, Burkholder BD, White DM, Tarka SM. Subchronic effects of feeding graded levels of cocoa butter to rats. *Nutr Res.* 1986;6(3):319–26. [https://doi.org/10.1016/S0271-5317\(86\)80135-X](https://doi.org/10.1016/S0271-5317(86)80135-X)
15. Kiitchevsky D, Tepper SA, Lloyd LM, Davidson LM, Klurfeld DM. Serum and liver lipids of rats fed cocoa butter, corn oil, palm kernel oil, coconut oil and cholesterol. *Nutr Res.* 1988;8(3):287–94. [https://doi.org/10.1016/S0271-5317\(88\)80046-0](https://doi.org/10.1016/S0271-5317(88)80046-0)
16. Kamesh VK, Sumathi T. Antihypercholesterolemic effect of *Bacopa monnieri* Linn on high cholesterol diet-induced hypercholesterolemia in rats. *Asian Pac J Trop Med.* 2012;949–955. [doi:10.1016/s1995-7645\(12\)60180-1](https://doi:10.1016/s1995-7645(12)60180-1)
17. American Institute of Nutrition (AIN). Purified diet for laboratory rodent, final report. *J Nutr.*

- 1993;123:1939–1951.
<https://DOI:10.1093/in/123.11.1939>
18. Schermer S. The blood morphology of laboratory animals: case-control study. *Avicenna J Clin Med*. 1967;24(4):307-14. Cited by:
<https://www.sciepub.com/reference/431311>
 19. Chapman DG, Castilla R, Campbell JA. Evaluation of protein in food. I. A method for the determination of protein efficiency ratio. *Can J Biochem Physiol*. 1959;37:679–686.
<https://doi.org/10.1139/o59-074>
 20. Thomas L. Labor und Diagnose, 4th Ed. Marburg: Die Medizinische Verlagsgesellschaft; 1992. <https://www.flickr-werk.de/images/Auszug%20Labor%20und%20Diagnose%20-%208te%20Auflage.pdf>
 21. Young D. Effects of drugs on clinical laboratory tests. Pestaner L, editor. *Clin Chem*. 1975;21(5):1D-432D.
<https://DOI: 10.1177/000456329703400601>
 22. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem*. 1982 Oct;28(10):2077-80.
<https://pubmed.ncbi.nlm.nih.gov/6812986/>
 23. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin Chem*. 1977 May;23(5):882-4. PMID:192488.
<https://pubmed.ncbi.nlm.nih.gov/192488/>
 24. Lee RD, Nieman DC. Nutritional assessment. 2nd ed. St. Louis (MO): Mosby; 1996. Cited by
https://mkas.journals.ekb.eg/article_271303_61f40ecc1fd6e30f91e9db379eb827b8.pdf
 25. Mudhaffar SK. Atherogenic index of plasma (AIP) as a parameter in predicting cardiovascular risk in males compared to the conventional dyslipidemic indices (cholesterol ratios). *Karbala J Med*. 2013;6(1):1506–13.
https://www.researchgate.net/publication/269964677_Atherogenic_index_of_plasma_Atherogenic_index_of_plasma_AIP_as_a_parameter_in_predicting_cardiovascular_risk_in_males_compared_to_the_conventional_dyslipidemic_indices_cholesterol_ratios
 26. Bhardwaj S, Bhattacharjee J, Bhatnagar MK, Tyagi S. Atherogenic index of plasma, Castelli risk index and atherogenic coefficient: new parameters in assessing cardiovascular risk. *Int J Pharm Biol Sci*. 2013 Jul-Sep;3(3):359-64. Available from: <http://www.ijpbs.com>
 27. Olamoyegun MA, Oluyombo R, Asaolu SO. Evaluation of dyslipidaemia, lipid ratios, and atherogenic index as cardiovascular risk factors among semi-urban dwellers in Nigeria. *Ann Afr Med*. 2016;15(3):194–9.
<https://DOI:10.4103/1596-3519.194280>
 28. Aguilar EC, Queiroz MGMN, Oliveira DA. Serum lipid profile and hepatic evaluation in mice fed diet containing pequinut or pulp (*Caryocar brasiliense* Camb.). *Cienc Tecnol Aliment*. 2011;31(4):879–883.
<https://doi.org/10.1590/S0101-20612011000400008>
 29. Rorth M, Jensen PK. Determination of catalase activity by means of the Clark oxygen electrode. *Biochim Biophys Acta*. 1967;139(1):171-3.
[https://DOI: 10.1016/0005-2744\(67\)90124-6](https://DOI: 10.1016/0005-2744(67)90124-6)
 30. Guillén-Sans R, Guzmán-Chozas M. The thiobarbituric acid (TBA) reaction in foods: a review. *Crit Rev Food Sci Nutr*. 1998;38(4):315–30.
<https://DOI: 10.1080/10408699891274228>
 31. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. 1967;70:158–69.
<https://DOI:10.5555/URI:PII:0022214367900765>
 32. Clinica Chimica Acta. Chemical kits. 1980;105:147–172.
https://mkas.journals.ekb.eg/article_214620_266ece8658aa5a5d839177711d882bf4.pdf
 33. Huang XJ, Choi YK, Im HS, Yarimaga O, Yoon E, Kim HS. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. *Sensors (Basel)*. 2006;6(7):756-82.
<http://doi:10.3390/s6070756>
 34. Moss DW. Alkaline phosphatase isoenzymes. *Clin Chem*. 1982 Oct;28(10):2007-16. PMID:6751596.
<https://pubmed.ncbi.nlm.nih.gov/6751596/>
 35. Jacobs DS, Oxley DK, DeMott WR, editors. Laboratory test handbook concise with disease index. 2nd ed. Hudson (Cleveland), OH: Lexi-Comp; 2002. 1348 p. ISBN: 1-930598-

- 50-5. doi:10.1373/49.1.205.
<http://doi.10.1373/49.1.205>
36. Bancroft JD, Stevens A. Theory and Practice of Histological Techniques. 4th ed. New York: Churchill Livingstone; 1996.
[https://doi.org/10.1002/\(SICI\)1096-9896\(199710\)183:2<243::AID-9896\(199710\)183:2>2.0.CO;2-D](https://doi.org/10.1002/(SICI)1096-9896(199710)183:2<243::AID-9896(199710)183:2<243::AID-9896(199710)183:2>2.0.CO;2-D)
37. Statistical Analysis System (SAS) Institute. SAS/STAT user's guide. Version 8. 6th ed. Cary (NC): SAS Institute; 2002. p.112.
<https://www.scrip.org/reference/References/Papers?ReferenceID=1139542>
38. Miller KB, Stuart DA, Smith NL, Lee CY, McHale NL, Flanagan JA, et al. Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States. J Agric Food Chem. 2006;54(11):4062-8.
<https://DOI:10.1021/jf060290o>
39. Halib H, Ismail A, Yusof BNM, Osakabe N, Daud ZM. Effects of cocoa polyphenols and dark chocolate on obese adults: A scoping review. Nutrients. 2020;12(12):3695.
<https://DOI:10.3390/nu12123695>
40. Greenberg JA, O'Donnell R, Shurpin M, Kordunova D. Epicatechin, procyanidins, cocoa, and appetite: A randomized controlled trial. Am J Clin Nutr. 2016;104(3):613-619.
<https://DOI:10.3945/ajcn.115.129783>
41. Massolt ET, van Haard PM, Rehfeld JF, Postuma EF, van der Veer E, Schweitzer DH. Appetite suppression through smelling of dark chocolate correlates with changes in ghrelin in young women. Regul Peptides. 2010;161(1-3):81-6.
<https://DOI:10.1016/j.regpep.2010.01.005>
42. Min SY, Yang H, Seo SG, Shin SH, Chung MY, Kim J, et al. Cocoa polyphenols suppress adipogenesis in vitro and obesity in vivo by targeting insulin receptor. Int J Obes. 2013;37:584-92.
<https://DOI: 10.1038/ijo.2012.85>
43. Chatree S, Sitticharoon C, Maikaew P, Pongwattanapakin K, Keadkraichaiwat I, Churintaraphan M, et al. Epigallocatechin gallate decreases plasma triglyceride, blood pressure, and serum kisspeptin in obese human subjects. Exp Biol Med (Maywood). 2021;246(2):163-176.
<https://DOI:10.1177/1535370220962708>
44. Rusdiana R, Syarifah S, Pane YS, Widjaja SS, Anggraini DR. The Effects of High Fat Diet on the Liver of the White Rat Model Obesity. Open Access Maced J Med Sci. 2022;10(A):709-14.
<https://DOI:10.3889/oamjms.2022.9383>
45. Rospond B, Krakowska A, Krośniak M, Muszyńska B, Opoka W. The influence of high-fat and high-sucrose feeding regimes on organ weight, body weight, and serum concentration of bioelements in rats. J Trace Elem Med Biol. 2022;73:127020.
<https://DOI: 10.1016/j.jtemb.2022.127020>
46. Ratri PR, Yulianti A, Restuti ANS. The effect of chocolate drink to hepatosomatic index of diabetes mellitus induced rat. IOP Conf Ser Earth Environ Sci. 2021;672(1):012074.
<https://DOI:10.1088/1755-1315/672/1/012074>
47. Nwichi SO, Adewole EK, Dada AO, Ogidiama O, Mokobia OE, Farombi EO. Cocoa powder extracts exhibits hypolipidemic potential in cholesterol-fed rats. Afr J Med Med Sci. 2012 Dec;41(Suppl):39-49. PMID:23678635.
<https://pubmed.ncbi.nlm.nih.gov/23678635/>
48. Allotey-Babington L, Kwamong AA, Banga KBNG, Amponsah SK, Asiedu-Gyekye JJ. Unsweetened natural cocoa powder: A potent nutraceutical in perspective. In: Theobroma Cacao—Deploying Science for Sustainability of Global Cocoa Economy. London: IntechOpen; 2019. <https://DOI:10.5772/intechopen.88145>
49. Fadlalla EAS, Faid SM. The effect of cocoa powder, white chocolate and dark chocolate on oxidative stress and lipid profile on hypercholesterolemic rats. J Am Sci. 2015;110-122.
https://www.jofamericanscience.org/journals/am-sci/am110415/012_28176am110415_110_12_2.pdf
50. Asiedu-Gyekye JJ, Edem Kukuia KK, Seidu AM, Antwi-Boasiako C, N'guessan BB, Frimpong-Manso S, et al. Unsweetened natural cocoa powder has the potential to attenuate high-dose artemether-lumefantrine-induced hepatotoxicity in non-malarious guinea pigs. Evid Based Complement Alternat Med. 2016;2016:7387286.
<https://DOI: 10.1155/2016/7387286>
51. Yasuda A, Natsume M, Sasaki K, Baba S, Nakamura Y, Kanegae M, Nagaoka S. Cacao

- procyanidins reduce plasma cholesterol and increase fecal steroid excretion in rats fed a high-cholesterol diet. *BioFactors*. 2008;33(3):211–23.
<https://DOI:10.1002/biof.5520330307>
52. Suyama K, Watanabe M, Sakabe K, Otomo A, Okada Y, Terayama H, et al. GRP78 suppresses lipid peroxidation and promotes cellular antioxidant levels in glial cells following hydrogen peroxide exposure. *PLoS One*. 2014;9(1):e86951.
[https://DOI: 10.1371/journal.pone.0086951](https://DOI:10.1371/journal.pone.0086951)
 53. Schneider CL, Cowles RL, Stuefer-Powell CL, Carr TP. Dietary stearic acid reduces cholesterol absorption and increases endogenous cholesterol excretion in hamsters fed cereal-based diets. *J Nutr*. 2000;130(5):1232–38.
<https://DOI:10.1093/jn/130.5.1232>
 54. Stonehouse W, Benassi-Evans B, James-Martin G, Abeywardena M. Fatty acid regio-specificity of triacylglycerol molecules may affect plasma lipid responses to dietary fats—a randomized controlled crossover trial. *Eur J Clin Nutr*. 2020;74(2):268–77.
[https://DOI: 10.1038/s41430-019-0452-7](https://DOI:10.1038/s41430-019-0452-7)
 55. Corti R, Flammer AJ, Hollenberg NK, Lüscher TF. Cocoa and cardiovascular health. *Circulation*. 2009;119(10):1433–1441.
[https://DOI: 10.1161/CIRCULATIONAHA.108.827022](https://DOI:10.1161/CIRCULATIONAHA.108.827022)
 56. Ouimet M, Barrett TJ, Fisher EA. HDL and reverse cholesterol transport: Basic mechanisms and their roles in vascular health and disease. *Circ Res*. 2019;124(10):1505–18.
[https://DOI: 10.1161/CIRCRESAHA.119.312617](https://DOI:10.1161/CIRCRESAHA.119.312617)
 57. Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH. The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. *J Lipid Res*. 2009;50 Suppl:S189–94.
<https://DOI:10.1194/jlr.R800088-JLR200>
 58. Nurhafsah Hanuddin R, Andriani I, Fitriawaty, Balai Pengkajian Teknologi Pertanian, Sulawesi Barat, Jurusan Teknologi Hasil Pertanian, et al. ultas Pertanian Universitas Ichsan Gorontalo, & Program Studi Gizi, Sekolah Tinggi Ilmu Kesehatan Partamedika. Pengaruh komposisi nibs kakao terhadap warna, kadar asam dan penerimaan panelis pada produk coklat susu. 2021.
<https://DOI:10.33104/jihp.v16i1.6861>
 59. Mackenbach JP. The temptations of chocolate. *BMJ (Clin Res Ed)*. 2011;343:d5883.
<https://DOI:10.1136/bmj.d5883>
 60. Kris-Etherton PM. AHA Science Advisory. Monounsaturated fatty acids and risk of cardiovascular disease. *Am Heart Assoc Nutr Committee. Circ*. 1999;100(11):1253–58.
<https://DOI:10.3390/nu4121989>
 61. Cleeman JI. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA*. 2001;285(19):2486–2497.
<https://DOI:10.1001/jama.285.19.2486>
 62. Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr*. 2003;77(5):1146–
<https://DOI:10.1093/ajcn/77.5.1146>
 63. Sokpor G, Addai FK, Gyasi RK, Bugyei KA, Ahenkorah J, Hottor B. Voluntary ingestion of natural cocoa extenuated hepatic damage in rats with experimentally induced chronic alcoholic toxicity. *Functional Foods in Health and Disease*. 2012;2(5):166–87.
<https://DOI:10.31989/ffhd.v2i5.91>
 64. Kilicgun H, Altiner D. The antioxidant activity of cocoa. *Pharmacogn Mag*. 2009;5(20).
<https://DOI:10.4103/0973-1296.58148>
 65. Aidoo E, Addai FK, Ahenkorah J, Hottor B, Bugyei KA, Gyan BA. Natural cocoa ingestion reduced liver damage in mice infected with *Plasmodium berghei* (NK65). *Res Rep Trop Med*. 2012;107–116.
<https://DOI:10.2147/RRTM.S33149>
 66. Asiedu-Gyekye IJ, Frimpong-Manso S, N'guessan BB, Seidu MA, Osei-Prempeh P, Boamah DK. Macro- and microelemental composition and toxicity of unsweetened natural cocoa powder in Sprague-Dawley rats. *J Toxicol*. 2016;2016:4783829.
<https://doi:10.1155/2016/4783829>
 67. Saleh AAG, Sabahelkhier MK. Impact of raw cocoa and chocolate on lipid profile and liver function in alcohol-induced toxicity Wister

- rats. 2017.
<https://doi.org/10.14419/IJM.V5I2.8321>
68. Schwenger KJ, Allard JP. Clinical approaches to non-alcoholic fatty liver disease. *World J Gastroenterol.* 2014;20(7):1712-23.
<https://DOI:10.3748/wjg.v20.i7.1712>
 69. McKim SE, Konno A, Gäbele E, Uesugi T, Froh M, Sies H, et al. Cocoa extract protects against early alcohol-induced liver injury in the rat. *Arch Biochem Biophys.* 2002;406(1):40–6.
[https://DOI:10.1016/S0003-9861\(02\)00425-3](https://DOI:10.1016/S0003-9861(02)00425-3)
 70. Chang HY, Chen JR, Chen YH, Xiao Q, Chen YL, Yang SC. Preliminary results for evaluating cocoa butter's hepatoprotective effects against lipid accumulation and inflammation in adult male rats chronically fed ethanol. *Bioengineering.* 2022;9(10):526.
<https://doi.org/10.3390/bioengineering9100526>
 71. Prenetha P, Geetha R, Parameswari R, Lakshmi T. In vitro antioxidant and cytochrome P450 inhibitory activity of dark chocolate mediated zinc oxide nanoparticles. *J Complement Med Res.* 2022;13(5):69.
<https://DOI:10.5455/jcmr.2022.13.05.13>
 72. S. Ahmed. Gpx and MDA Oxidative Stress Markers and Severity of Depression as Predictives of Recurrent Stable Coronary Heart Disease. Doctoral Thesis. 2023.
https://doi:10.25143/prom-rsu_2023-03_dt
 73. Ramos-Romero S, Pérez-Cano FJ, Ramiro-Puig E, Franch A, Castell M. Cocoa intake attenuates oxidative stress associated with rat adjuvant arthritis. *Pharmacol Res.* 2012;66(3):207–12.
<https://DOI:10.1016/j.phrs.2012.05.009>
 74. Jenny M, Santer E, Klein A, Ledochowski M, Schennach H, Ueberall F, et al. Cacao extracts suppress tryptophan degradation of mitogen-stimulated peripheral blood mononuclear cells. *J Ethnopharmacol.* 2009;122:261–267.
<https://DOI:10.1016/j.jep.2009.01.011>
 75. Parsaeyan N, Mozaffari-Khosravi H, Absalan A, Mozayan MR. Beneficial effects of cocoa on lipid peroxidation and inflammatory markers in type 2 diabetic patients and investigation of probable interactions of cocoa active ingredients with prostaglandin synthase-2 (PTGS-2/COX-2) using virtual analysis. *J Diabetes Metab Dis.* 2014;13(1):30.
<https://DOI:10.1186/2251-6581-13-30>
 76. Ramirez-Sanchez I, Taub PR, Ciaraldi TP, et al. (-)-Epicatechin rich cocoa mediated modulation of oxidative stress regulators in skeletal muscle of heart failure and type 2 diabetes patients. *Int J Cardiol.* 2013;168:3982-90.
<https://DOI:10.1016/j.ijcard.2013.06.089>
 77. Kris-Etherton PM, Keen CL. Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. *Curr Opin Lipidol.* 2002;13(1):41–9.
<https://DOI:10.1097/00041433-200202000-00007>
 78. Shaheen K, Youssef H, Khafagy M, Rashed F. The potential effect of cocoa powder on serum lipids profile and antioxidant status in hypercholesterolemic rats. *J Home Econ-Menofia Univ.* 2016;26(3):337-348.
https://mkas.journals.ekb.eg/article_170693_315de7eb9ea8fb2b7f17643933b876d2.pdf
 79. Middendorf D, Juadjur A, Bindrich U, Mischnick P. AFM approach to study the function of PGPR's emulsifying properties in cocoa butter-based suspensions. *Food Struct.* 2015;4:16-26.
<https://doi:10.1016/j.foostr.2014.11.003>
 80. Abrokwhah FK, Asamoah KA, Esubonteng PK. Effects of the intake of natural cocoa powder on some biochemical and haematological indices in the rat. *Ghana Med J.* 2009 Dec;43(4):164-8. Available from: <http://www.ghanamedj.org/articles/December2009/Final%20intake%20of%20natural%20cocoa%20powder.pdf>
 81. Stobbs JA, Pensini E, Ghazani SM, Leontowich AF, Quirk A, Tu K, et al. Phospholipid self-assembly in cocoa butter provides a crystallizing surface for seeding the form V polymorph in chocolate. *Crystal Growth & Design.* 2024;24(7):2685-99.
<https://DOI:10.1021/acs.cgd.3c01130>
 82. Zięba K, Makarewicz-Wujec M, Kozłowska-Wojciechowska M. Cardioprotective mechanisms of cocoa. *J Am Coll Nutr.* 2019;38(6):564-575.
<https://DOI:10.1080/07315724.2018.1557087>
 83. Grassi D, Mai F, De Feo M, Barnabei R, Carducci A, Desideri G, et al. Cocoa consumption decreases oxidative stress, proinflammatory mediators and lipid peroxidation in healthy subjects: A

- randomized placebo-controlled dose-response clinical trial. *High Blood Press Cardiovasc Prev.* 2023;30(3):219–225. <https://DOI:10.1007/s40292-023-00571-8>
84. Arranz S, Valderas-Martinez P, Chiva-Blanch G, Casas R, Urpi-Sarda M, Lamuela-Raventos RM, et al. Cardioprotective effects of cocoa: Clinical evidence from randomized clinical intervention trials in humans. *Mol Nutr Food Res.* 2013;57(6):936–947. <https://DOI:10.1002/mnfr.201200595>
 85. Patel K, Watson RR. Chocolate and its component's effect on cardiovascular disease. In: *Lifestyle in Heart Health and Disease.* 2018:255–66. <https://DOI:10.1016/B978-0-12-811279-3.00021-5>
 86. Buitrago-Lopez A, Sanderson J, Johnson L, Warnakula S, Wood A, Di Angelantonio E, et al. Chocolate consumption and cardiometabolic disorders: Systematic review and meta-analysis. *BMJ.* 2011;343:d4488. <https://DOI:10.1136/bmj.d4488>
 87. Haber SL, Gallus K. Effects of dark chocolate on blood pressure in patients with hypertension. *Am J Health Syst Pharm.* 2012;69:1287–1293, 1292–1293. <https://DOI:10.2146/ajhp110498>
 88. Vinson JA, Proch J, Bose P, Muchler S, Taffera P, Shuta D, et al. Chocolate is a powerful ex vivo and in vivo antioxidant, an antiatherosclerotic agent in an animal model, and a significant contributor to antioxidants in the European and American Diets. *J Agric Food Chem.* 2006;54(21):8071–6. <https://DOI:10.1021/jf062175j>
 89. Jalil AM, Ismail A. Polyphenols in cocoa and cocoa products: Is there a link between antioxidant properties and health? *Molecules.* 2008;13(9):2190–2219. <https://doi:10.3390/molecules13092190>
 90. Cooper KA, Donovan JL, Waterhouse AL, Williamson G. Cocoa and health: A decade of research. *Br J Nutr.* 2008;99(1):1–11. <https://DOI:10.1017/S0007114507795296>
 91. Garcia JP, Santana A, Baruqui DL, Suraci N. The cardiovascular effects of chocolate. *Rev Cardiovasc Med.* 2018;19(4). <https://DOI:10.31083/j.rcm.2018.04.3187>
 92. Eagappan K, Sasikumar S. Cardioprotective effect of dark chocolate components: Mechanisms of actions. *Cardiol Angiol Int J.* 2014;163–173. <https://DOI:10.9734/CA/2014/6835>
 93. Yokoi K, Konomi A, Otagi M. Iron bioavailability of cocoa powder as determined by the Hb regeneration efficiency method. *Br J Nutr.* 2008;102(2):215–20. <https://DOI:10.1017/S0007114508149182>
 94. Yokoi K, Konomi A, Otagi M. Comparison of a modified hemoglobin regeneration efficiency method with a slope-ratio assay in measuring relative bioavailability of cocoa powder iron using rats. *Biol Trace Elem Res.* 2011;143:1103–9. <https://DOI:10.1007/s12011-010-8953-1>
 95. Zhu QY, Schramm DD, Gross HB, Holt RR, Kim SH, Yamaguchi T, et al. Influence of cocoa flavanols and procyanidins on free radical-induced human erythrocyte hemolysis. *J Immunol Res.* 2005;12(1):27–34. <https://DOI:10.1080/17402520512331329514>
 96. Heptinstall S, May J, Fox S, Kwik-Urbe C, Zhao L. Cocoa flavanols and platelet and leukocyte function: Recent in vitro and ex vivo studies in healthy adults. *J Cardiovasc Pharmacol.* 2006;47:S197–S205. <https://DOI:10.1097/00005344-200606001-00015>
 97. Kenny TP, Shu SA, Moritoki Y, Keen CL, Gershwin ME. Cocoa flavanols and procyanidins can modulate the lipopolysaccharide activation of polymorphonuclear cells in vitro. *J Med Food.* 2009;12(1):1–7. <https://DOI:10.1089/jmf.2007.0263>
 98. Peluso I, Palmery M, Serafini M. Effect of cocoa products and flavanols on platelet aggregation in humans: a systematic review. *Food Funct.* 2015;6(7):2128–34. <https://DOI:10.1039/C5FO00113G>
 99. Seecheran NA, Sukha D, Grimaldos K, Grimaldos G, Richard S, Ishmael A, et al. Effect of cocoa (*Theobroma cacao* L.) on platelet function testing profiles in patients with coronary artery disease: ECLAIR pilot study. *Open Heart.* 2022;9(2):e002066. <https://DOI:10.1136/openhrt-2022-002066>
 100. Kim SJ, Park SH, Lee HW, Schini-Kerth VB, Kwon O, Lee KW, Oak MH. Cacao polyphenols potentiate anti-platelet effect of endothelial cells and ameliorate hypercoagulatory states associated with hypercholesterolemia. *J Nanosci Nanotech.* 2017;17(4):2817–23. <https://doi.org/10.1166/jnn.2017.13317>

101. Addai F. Need to introduce cocoa products in basic schools. *Mod Ghana News*. 2009;217980.
102. Abrokwah FK, Asamoah KA, Esubonteng PK. Effects of the intake of natural cocoa powder on some biochemical and haematological indices in the rat. *Ghana Med J*. 2009 Dec;43(4):164-8. <https://pubmed.ncbi.nlm.nih.gov/21326996/>
103. Yamamoto, Tsunehisa, et al. Decrease in membrane phospholipids unsaturation correlates with myocardial diastolic dysfunction. *PLoS One*, 2018, 13.12: e0208396. <https://DOI:10.1371/journal.pone.0208396>
104. Calligaris, Sebastian D., et al. Mice long-term high-fat diet feeding recapitulates human cardiovascular alterations: an animal model to study the early phases of diabetic cardiomyopathy. *PloS one*, 2013, 8.4: e60931. <https://DOI:10.1371/journal.pone.0060931>
105. Nguyen S, Shao D, Tomasi LC, Braun A, de Mattos ABM, Choi YS, et al. The effects of fatty acid composition on cardiac hypertrophy and function in mouse models of diet-induced obesity. *J Nutr Biochem*. 2017;46:137–42. <https://DOI:10.1016/j.jnutbio.2017.05.009>
106. Grassi D, Desideri G, Mai F, Martella L, De Feo M, Soddu D, et al. Cocoa, glucose tolerance, and insulin signaling: cardiometabolic protection. *J Agric Food Chem*. 2015;63(45):9919–26. <https://DOI:10.1021/acs.jafc.5b00913>
107. Stote KS, Clevidence BA, Novotny JA, Henderson T, Radecki SV, Baer DJ. Effect of cocoa and green tea on biomarkers of glucose regulation, oxidative stress, inflammation and hemostasis in obese adults at risk for insulin resistance. *Eur J Clin Nutr*. 2012;66(10):1153–9. <https://DOI:10.1038/ejcn.2012.101>
108. Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. *Antioxid Redox Signal*. 2011;15(10):2779–811. <https://DOI:10.1089/ars.2010.3697>
109. Kandhi R, Menendez A, Ramanathan S, Ilangumaran S. Regulation of high-fat diet-induced liver fibrosis by SOCS1 expression in hepatic stellate cells. *J Clin Exp Hepatol*. 2024;14(1):101280. <https://doi.10.1016/j.jceh.2023.09.001>
110. Park SR, Cho CS, Xi J, Kang HM, Lee JH. Holistic characterization of single-hepatocyte transcriptome responses to high-fat diet. *Am J Physiol Endocrinol Metab*. 2021;320(2):E244–E258. <https://doi:10.1152/ajpendo.00391.2020>
111. Rebollo-Hernanz M, Aguilera Y, Martin-Cabrejas MA, Gonzalez de Mejia E. Phytochemicals from the Cocoa Shell Modulate Mitochondrial Function, Lipid and Glucose Metabolism in Hepatocytes via Activation of FGF21/ERK, AKT, and mTOR Pathways. *Antioxidants* (Basel). 2022;11(1):136. <https://DOI:10.3390/antiox11010136>
112. Sun M, Gu Y, Glisan SL, Lambert JD. Dietary cocoa ameliorates non-alcoholic fatty liver disease and increases markers of antioxidant response and mitochondrial biogenesis in high fat-fed mice. *J Nutr Biochem*. <https://DOI:10.1016/j.jnutbio.2021.108618>
113. Divya V, Baskaran D. Characterization of thermal behaviour and fatty acid profile of coconut-based chocolate novelties. *Asian J Dairy Food Res*. 2020;OF. <https://DOI:10.18805/ajdfr.DR-1514>
114. Rodríguez Furlán LT, Baracco Y, Zaritzky N, Campderrós ME. Development of free sugar white chocolate, suitable for diabetics, using Stevia and sucralose as sweeteners: study of the thermal degradation kinetic. *Int J Res Advent Technol*. 2016 Jul;4(7):49-55. Available from: <http://www.ijrat.org>



JHE

مجلة الاقتصاد المنزلي، جامعة المنوفية

<https://mkas.journals.ekb.eg>
الترقيم الدولي اون لايين الترقيم الدولي للطباعة
2735-5934 2735-590X

تاريخ الاستلام: ١٩ ديسمبر ٢٠٢٤
تاريخ القبول: ٢٠ يناير ٢٠٢٥
تاريخ النشر: ١ ابريل ٢٠٢٥

نوع المقالة: بحوث أصلية
التغذية وعلوم الاطعمة

التأثيرات المحتملة للشوكولاتة البيضاء والداكنة في الفئران المصابة بارتفاع كوليسترول الدم

اسراء أبو شادي، شريف رجب، سماح البنا

قسم التغذية وعلوم الأطعمة، كلية الاقتصاد المنزلي، جامعة المنوفية، شبين الكوم، مصر.
* المؤلف المسئول: اسراء أبو شادي - البريد الإلكتروني: esraashady0@gmail.com

الملخص العربي:

الهدف من البحث هو دراسة تأثير الشوكولاتة الداكنة والشوكولاتة البيضاء على بعض المعاملات البيولوجية والبيوكيميائية والنسجية لدى الفئران المصابة بفرط كوليستيرول الدم. تم توزيع ستة وثلاثين فأر من ذكور الفئران البيضاء البالغة على مجموعتين رئيسيتين. المجموعة الرئيسية الأولى تم تغذيتها على النظام الغذائي الأساسي كمجموعة ضابطة سالبة. تمت إصابة المجموعة الرئيسية الثانية عن طريق إطعام نظام غذائي عالي الكوليستيرول [4% كوليستيرول و 1% حمض الكوليك] لمدة 45 يوماً مقسمة إلى خمس مجموعات فرعية، المجموعة الفرعية الأولى تغذت على نظام غذائي أساسي كمجموعة ضابطة موجبة. كانت المجموعات الفرعية الأربع الأخرى عبارة عن فئران مصابة بفرط كوليستيرول الدم وتم إعطاؤها الشوكولاتة الداكنة بنسبة 5 و 10% والشوكولاتة البيضاء بنسبة 5 و 10% على التوالي لمدة 28 يوماً. أظهرت النتائج انخفاضاً معنوياً في معظم مؤشرات دهون الدم ووظائف الكبد ومضادات الأكسدة ومعاملات دهون الدم. من ناحية أخرى أظهرت النتائج ارتفاعاً معنوياً في بعض مؤشرات ملف دهون الدم ومضادات الأكسدة ومعاملات الدم. وتم تدعيم النتائج البيوكيميائية بالفحص النسيجي. لذا أوصت الدراسة بتناول الشوكولاتة الداكنة بكمية معتدلة في النظام الغذائي

الكلمات الكاشفة: فرط كوليستيرول الدم – كوليستيرول – الشوكولاتة الداكنة – الشوكولاتة البيضاء..

الاستشهاد الي:

Abo Shady et al., 2025, Potential Effects of White and Dark Chocolate in Hypercholesterolemic Rats. 35 (2), 151-172.
DOI: 10.21608/mkas.2025.345883.1354