Journal of Home Economics

Volume 26, Number (3), 2016

http://homeEcon.menofia.edu.eg

Journal of Home Economics

ISSN 1110-2578

The Potential Effect of cocoa powder on serum lipids profile and antioxidant status inhypercholesterolemic rats

Khaled Shaheen, Heba Youssef, Mai Khafagy and FatenRashed
Nutrition and Food Science Department, Faculty of Home Economics
MenofiaUniversity,shebin el-kom,egypt

Abstract

The present investigation aims to evaluate the effect of cocoa powder on serum lipids profile and antioxidant status in normal and hypercholesterolemic rats. Twenty four adult albino Rats were randomly divided into two main groups. Group I: negative control (6 rats) was fed on standard diet . Group II: hypercholesterolemic groups (18 rats) were divided into equal three sub groups, the first: positive control group, the second and the third groups were fed on standard diet containing 2.5 and 5% of cocoa powder respectively. The results indicated that the consumption of cocoa led to decrease the levels of blood cholesterol,LDLc and VLDLc as compared with the positive control group. On the other hand, the treatments significantly $p \leq 0.05$ increased the glutathione transferase and catalase level activities compared with the positive control group, while the malonaldehyde content had the opposite trend. In conclusion, this study indicated that cocoa used effectively in reducing the levels of lipid profile in hypercholesterolemic rats.

Keywords: cocoa, hyperlipidemia ,flavanoids, lipid profile.

Introduction

Hypercholesterolemia is the presence of high levels of cholesterol in the blood **(Durrington,2003).** The high low density lipoprotein cholesterol (LDLc) level frequently gives rise to xanthiums, deposits of

cholesterol in peripheral tissues, and accelerated atherosclerosis resulting from cholesterol deposition in the arterial wall that can lead to accelerated atherosclerosis and premature cardiovascular disease (**Soutarand noumara , 2006)**. Typically, approximately 45% of male and 20% of female patients suffer from coronary artery disease (CAD) by the age of 50 (**Emily *et al*., 2006).**

Cocoa is widely consumed as chocolates and in desserts, and used to produce beverages, cosmetics, pharmaceuticals and toiletries (**Castell *et al*., 2015)**.Cocoa products are major sources of antioxidants, which have been shown to have protective effects against heart disease (**Ding *et al*., 2006).** It has been known for its good taste and its beneficial effects on health. The health benefits associated with cocoa consumption have been related to their protective effect mainly against cardiovascular diseases, but also in other diseases such as age-related cognitive decline **(Castell, 2015)**.These effects are due to antiradical properties of cocoa phenolics which increase the plasma level of antioxidants to prevent the oxidation of LDL-cholesterol (**Andújar *et al*.,** 2012**)**.Also cocoa polyphenols (flavanols) have been reported to have a wide range of biological properties including modulating eicosanoid synthesis, increasing nitric oxide synthesis, lowering the rate of LDL-C oxidation, inhibiting platelets activation, stimulating the production of antiinflammatory cytokines among others**(Wollgast and Anklain, 2000**). The present study aims to evaluate the effect of cocoa powder on serum lipids profile, liver, kidney function and antioxidant status on hypercholesterolemic rats.

Materials and methods

Materials

The cocoa beans (*Theobroma cacao L.)* were obtained from Haraz shops Cairo, Egypt. All analysis kits were purchased from Bio diagnostic Co., Giza, Egypt. Other chemicals used throughout the experiments were purchased from El-Nasr Pharmaceutical Chemicals, El-Ameriea, Cairo, Egypt.Twenty four adult albino male rats Sprague – Dawley strain were purchased from the Medical Insects Research Institute, Doki, Cairo, Egypt.

Methods

Preparation of cocoa powder

Cocoa beans were cleaned manualy to remove foreign materials. The beans were dried at 40°C in an electric draught oven and ground to pass through a 60 mesh sieve then kept in cold for analysis.

Chemical composition and bioactive compounds of cocoa powder

Moisture, fat, protein, fiber and ash contents were determined in cocoa powder according to **AOAC, (2005)**. The carbohydrate was calculated by difference. The antioxidant activity and the total phenolic of cocoa powder were determined according to **Velioglu *et al* ., (1998)** and**oband and owuor , (1997)** respectively**.**

Biological investigations

Induction ofhypercholesterolemic rats

Eighteen rats fed on hypercholesterolemic diet (1.5 % cholesterol and 5% tallow) for 3 weeks, then fasting blood serum obtained, total cholesterol (TC), triglyceride (TG), (HDL-C), (LDL-C) and very low density lipoprotein (VLDL-C), levels estimated.

Experimental design

Adult male rats Sprague Dawley weighting (150±5 g) were used in this study. The animals were housed individually in well aerated cages under hygienic laboratory condition and fed standard diet according to **AIN-93** guidelines (**Reeves *et al*., 1993**) for 7 days as an adaptation period.The standard diet comprised of casein 85% (120 g/kg), corn starch (677g/kg), cellulose (50g/kg), corn oil (100 g/kg), mineral mixture (40g/kg), vitamins mixture (10g/kg) and DL-methionine (3g/kg).Rats were randomly divided into two main groups and fed standard diet. Group I: negative control (6 rats). Group II: hypercholesterolemic groups (18 rats) which were divided into equal three sub groups, the first: positive control group and the second and the third group fed on standard diet containing 2.5 and 5%of cocoa powder, respectively.

Blood sampling

At the end of the experimental period (30 days), rats were anesthetized with diethyl ether after fasting for 12h and blood samples were collected by using the retro-orbital method by means of a micro capillary glass tubes. Blood was collected into a dry clean centrifugal

tube and left to clot in a water bath (37°C) at room temperature for half an hour. The blood was centrifuged for 10 minutes at 3000 rpm to separate the serum. Serum was kept in clear quit fit plastic tubes and stored at -20°C until analysis.

Biochemical analysis

Alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) enzymes activity were measured according to the methods described by **Bergmeyer and Harder (1986), Kachmar and Moss (1976) and Varley*et al*., (1980),** respectively. Urea and creatinine levels were determined according to the method described by **Houot (1985)**. Catalase (CAT), glutathione transferase (GTH) and malonaldehyde (MDA) were determined according to the methods described by **Hu (1994), Aebi, (1974) and Jentzsch*et al*., (1996)** respectively**.** Total cholesterol (TC), triglycerides (T.G) and (HDL-c) were determined according to**Allain,(1974) , Fossati and Prencipe, (1982)** and **Schermer, (1967)** respectively**.** Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated according to the methods of **Lee and Nieman (1996)** as follows:

VLDL=TG/5.

LDL=(Total cholesterol)- (HDL+VLDL).

Statistical Analysis

The results recorded as the mean ± SD. The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system (**SPSS, 1988)**. Duncan's multiple range tests were used to determine the differences among means at the level of 5%.

Results and discussion

Table (1) showed the chemical composition, total phenols and antioxidant activity of cocoa powder. The chemical compositions of cocoa powder were 2.63,48.82,36.71,8.95,2.89 and 11.66 g/100 for moisture, carbohydrate, fat, protein, ash and fiber respectively**.** These findings are in accordance with **Oliveira and Genovese, (2013**) who reported that the chemical compositions in cocoa were moisture(2.3–2.6%), lipid (52–54%), and fiber (27–32%) contents of cocoa.

Castell *et al*., (2015) showed that cocoa powder isconsidered a good source of fiber (26%–40%), proteins (15%–20%), carbohydrates (about

15%) and lipids (10%–24%). Also the results indicated that cocoa powder had total phenols (375.6 mg/100g) andantioxidant activity (35.74%).

Data presented in Table (2) show the effect of cocoa powder on serum lipids profile of normal and hypercholesterolemic rats. There were significant differences (P≤0.05) in total cholesterol (TC), triglycerides (T.G), high density lipoprotein (HDL),low density lipoprotein (LDL) and very low density lipoprotein (VLDL) values between negative and hypercholesterolemic rats. Supplementation of diet rats with 2.5 and 5% of cocoa powder resulted in reducing TC, TG, LDL and VLDL. Also no significant difference (p>0.05) was found in HDL between rats supplemented with 2.5% of cocoa powder and positive groups. These results are in the same trend of **Lecumberri *et al.*, (2007) and Kheder, (2011)** who found that consumption of cocoa fiber with a hypercholesterolemia diet improved the lipid profile. In the same table the results indicated that the highest reduction in TC, TG, LDL and VLDL and increasing in HDL were observed in rats supplemented with 5% of cocoa powder. This may be due to high phenolic compounds contents in rat diets supplemented with 5% of cocoa powder than 2.5% of cocoa powder.In the same content **Kurosawa *et al.*, (2005)** reported that ant-oxidative activity of polyphenols rich in cocoa may be a key factor for improving the lipids profile. Also **Jia *et al.*,(2010)** showed that polyphenols have been shown to inhibit cholesterol absorption, decreased serum lipid peroxidation, increased serum HDL cholesterol and decrease in LDL-C.

Effects of cocoa powder on liver functions of normal and hypercholesterolemic rats are shown in Table (3). Positive group had higher significant (P≤0.05) (ALT), (AST) and (ALP) activities than negative and rats supplemented with cocoa powder (5%) groups.These results are in agree with **Khedr, (2011)**who reported that there were significant(P≤0.05) increase in ALT, AST and ALP activities in positive control rats compared with rats supplemented with cocoa powder. Supplementation rat diets with 2.5 and 5% of CP did not differ in their effect on ALT AST and ALP. Also no significant (P>0.05) differences in AST and ALP activities between positive and rats supplemented with CP (2.5%) group. These results had the same trend

of**Abrokwah*et al.*,(2009)** who reviewed that administration of cocoa does not affect on liver functions (ALT, AST and ALP).

Effect of cocoa powder on the kidney functions of normal and hypercholesterolemic rats are shown in Table (4). No significant (P>0.05) differences in createnine and urea levels were observed between negative and hypercholesterolemic rats groups. However, negative control had significant lower (P≤0.05) uric acid than hypercholesterolemic rats. However there were no significant in uric acid between positive and rats supplemented with cocoa powder. These results agree with**Abrokwah *et al.*, (2009)** who found that administration rats with cocoa had no significant effect on uric acid.

Data in Table (5) indicated that the levels of CAT and GTF in positive control were significantly decreased after feeding on hypercholesterolemic diet ($p \leq 0.05$), while MDA had opposite trend. The decrease in the levels of CAT, GTF and the increase in MDA leveles may be due to the cause of the oxidative stress resulting from high blood fats and deposited in the walls of arteries. CAT and GTF were significantly ($p \leq 0.05$) increased by feeding rats with cocoa powder .These results are in agreement with **Ramiro-Puig*et al.*, (2007)** who found that cocoa diet enhances thymus antioxidant defenses and influences thymocyte differentiation. Also cocoa diet reduced reactive oxygen species (ROS) levels and carbonyl content in the liver **Castell *et al.*, (2015).** Moreover cocoa supplementation may enhance the antioxidant defense system **Jalil *et al.*, (2008)**.

Conclusion : The results suggest that cocoa powder had Hypercholesterolemic and antioxidant effect, which may be attributable to flavonoids contained in cocoa.

Table (1): Chemical composition, total phenolic and antioxidant activity of cocoa powder

Parameter	value
Moisture(g/100g)	**2.63 ±0.02**
Carbohydrate(g/100g)	**48.82±0.02**
Total fat (g/100g)	**36.71±0.4**
Protein (g/100g)	**8.95±0.05**
Ash (g/100g)	**2.89±0.04**
Fiber (g/100g)	**11.66±0.06**
Total phenols(mg/100g)	**375.6±0.4**
Antioxidant activity (%)	**35.74±0.04**

Table (2): Effect of cocoa powder on serum lipids profile of normal and hypercholesterolemic rats

Parameters	Negative group	Hypercholesterlemic groups		
		Positive	CP 2.5%	CP 5%
TC(mg/dl)	$122.0^{d}\pm2.6$	$184.0^{a}\pm3.6$	$136.67^{b}\pm1.5$	$129.8^{c}\pm1.6$
TG (mg/dl)	$95.0^{c}\pm2.0$	$130.0^{a}\pm5.0$	$112.67^{b}\pm2.5$	$104.33^{c}\pm2.5$
HDL (mg/dl)	$43.0^{a}\pm2.0$	$24.70^{c}\pm1.5$	$27.0^{c}\pm1.0$	$32.0^{b}\pm2.0$
LDL (mg/dl)	$60.0^{d}\pm1.2$	$133.33^{a}\pm2.1$	$87.03^{b}\pm0.2$	$76.93^{c}\pm3.9$
VLDL(mg/dl)	$19.0^{d}\pm0.4$	$26.0^{a}\pm1.0$	$22.53^{b}\pm0.5$	$20.86^{c}\pm0.5$

Values were expressed as means + SD. Different letters in the same row are significantly different (P≤0.05).

Table (3) :Effect of cocoa powder on liver functions of normal and hypercholesterolemic rats

Parameters	Negative group	Hypercholesterlemic groups		
		Positive	CP 2.5%	CP 5%
ALT(U/L)	$44.75^{b}\pm4.4$	$56.7^{a}\pm2.0$	$49.67^{b}\pm3.1$	$46.33^{b}\pm2.1$
AST (U/L)	$69.55^{c}\pm1.9$	$78.97^{a}\pm0.5$	$77.67^{ab}\pm3.05$	$72.5^{bc}\pm4.5$
ALP (U/L)	$114.67^{b}\pm3.8$	$126^{a}\pm6.5$	$124^{ab}\pm1.0$	$116.17^{ab}\pm7.2$

Values were expressed as means + SD. Different letters in the same row are significantly different (P≤0.05).

Table (4): Effect of cocoa powder on kidney functions of normal and hypercholesterolemic rats

Parameters	Negative group	Hypercholesterlemic groups		
		Positive	CP 2.5%	CP 5%
Creatinine(mg/dl)	$0.83^{a}\pm0.03$	$0.85^{a}\pm0.04$	$0.82^{a}\pm0.02$	$0.83^{a}\pm0.03$
Uric acid (mg/dl)	$3.22^{b}\pm0.3$	$3.79^{a}\pm0.2$	$3.58^{ab}\pm0.4$	$3.36^{ab}\pm0.1$
Urea (mg/dl)	$34.67^{a}\pm1.5$	$36.67^{a}\pm1.5$	$36.0^{a}\pm1.0$	$35^{a}\pm1.0$

Values were expressed as means + SD. Different letters in the same row are significantly different (P≤0.05).

Table (5):Effect of cocoa powder on oxidative status of normal and hypercholesterolemic rats

Parameters	Negative group	Hypercholesterlemic groups		
		Positive	CP 2.5%	CP 5%
MDA (nmol/dl)	30.08^{C}±1.9	41.82^{a}±0.5	36.49^{b}±1.0	25.49^{d} ±1.1
GTF(mg/dl)	989.63^{a} ±2.9	707.67^{c}±47.5	891.1^{b}±17.9	934.5^{ab} ±39.1
CAT(unit/prot)	29.13^{a}±1.6	15.8^{c} ±0.4	23.7^{b}±1.4	28.5^{a}±0.8

Values in the table were expressed as means + SD. Different letters in the same row are significantly different (P≤0.05).

Refrences

A.O.A.C. International official methods of analysis(2012):19th ed., Gaithersubrg, MD.

Abrokwah , F. K ., Asamoah,K. A., and Esubonteng , P .K., (2009) : Effects of the intake of natural cocoa powder on some Biochemical and Haematological Indices in the Rat **.,** Ghana Med JDec; 43(4): 164–168.

Aebi, H.(1974). Catalase In: Bergmeyer HV, editor. Methods in enzym atic analysis Vol 2, New York: Acadamic press, 17: 674-84.

AIN (1993): American Institute of Purified Diet for Laboratory Rodent, Final Report. J. Nutr. , 123:1939-1951.

Allain, C. C. (1974): Cholesterol enzymatic colorimetric Method. J. Clin. Chem., 20: 470.

Andújar , I. , Recio, M. C ., Giner, R. M., and **Ríos ,J. L.,** (2012) **:** Cocoa Polyphenols and Their Potential Benefits for Human Health , Department of Pharmacology, Faculty of Pharmacia, University of Valencia, Avanade Vincent Andrés Estelle's s/n, 46100 Burjassot, Valencia, Spain.

Bergmeyer, H. U., and Harder, M ., (1986). A colorimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase Clin. Biochem., 24: 481-486.

Castell ,M., Ruíz , S.S ., Lagunas ,M.M., Franch ' A ., and Cano,F.G., (2015) : Second International Congress on Chocolate and Cocoa in Medicine Held in Barcelona, Spain.,Nutrients 2015, *7*(12), 9785-9803

Ding , **E. L.** , **Hutfless**, **S. M.**, **Ding,X.,** and **Girotra** , **S., (** 2006) : Chocolate and Prevention of Cardiovascular Disease., Nutrition & Metabolism .7075-3-2.

Durring Ton ,T.P.,(2003):"Dyslipidaemia"lancet 362(9385): 717-31 .

Emily, S., van Aalst-Cohen., Angelique, C.M., Jansen , Michael, W.T., Tanck ., Joep, C., Defesche ., Mieke, D., Trip., Peter, J., Lansberg., Anton, F.H., Stalenhoef., John ,J.P., and Kastelein.,(2006) : Diagnosing familial hypercholesterolaemia: the relevance of genetic testing , European heart journal.

Fossati, P. and Prencipe, L. (1982): Triglyceride enzymatic colorimatric method. J. Clin. Chem., 28: 2077.

Houot, O., Bednawska, M.W., Zhiri, A., and Slest, G.(1985). Simultaneous Determination of Uric Acid and Creatinine in Plasma by Reversed-Phase Liquid Chromatography .Journal of Clinical Chemistry, 31(1):109.

Hu, M. L., (1994). Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol., 233: 380-385.

Jentzsch, A.M., Bachmann, H., Fürst, P and Biesalski, H.K,(1996). Improved analysis of malondialdehyde in human body fluids. Free RadicBiol Med.,20:251–256.

Jalil ,A.M., Ismail. A., Pei, C.P., Hamid ,M., and Kamaruddin, S.H.,(2008): Effects of cocoa extract on glucometabolism, oxidative stress, and antioxidant enzymes in obese-diabetic (Ob-bd) rats. J.Agric Food Chem.sep 10;56(17):7877-84.

Jia , L., Liu , X ., Bai,Y., Sun ,K ., Chen , H .,and Hui , R., (2010) : Short-term effect of cocoa product consumption on lipid profile: a meta-analysis of randomized controlled trials **.,** American Society for Nutrition.

Kachmar, J. F ., and Moss, D. W. (1976). Enzymes, In: Fundamentals of Clinical Chemistry (edited by Tiez N). pp. 666-672ph.d.thesis, Philadelphia PA. W.B. Saunders Co.

Khedr ,A.A ., (2011) :Effect of cocoa powder and its extracts on lipid profile Faculty of Home Economics, Menufyia University, Egypt

Kurosawa, T., Itoh, F., Nakano, Y., Nozaki, A., Katsuda, S., Osakabe, N., Tsubone, H., Konodo, K., Itakura, H.,(2005):

Suppressive effect of cocoa powder on atherosclerosis in kurosawaandKusanagihypercholesterolemic rabbits. J Atheroscler thromb;12(1):20-8.

Lecumberri, F., Goya, L., Matreos, R., Ali , M., Ramos S., Izquierdo-Pulido M., and Bravo, L., (2007). A diet rich in dietary fiber from cocoa improves lipid profile and reduces Malondialdehyde in hypercholesterolemia rats .Nutrition . 23(4):332-41 .

Lee, R., and Nieman, D., (1996): Nutrition Assessment. 2nd Ed. Mosby, Missouri, USA,. 591 – 594.

Obanda, M., and Owuor, P.O., (1997): Flavanol composition and caffeine content of green leaf as quality potential and aromatic plant extract. J. Sci. Food Agr. 74, 209– 215.

Oliveira, T.B , and Genovese, I.G ., (2013) : Chemical composition of cupuassu (Theobromagrandiflorum) and cocoa (Theobroma cacao) liquors and their effects on streptozotocin-induced diabetic rats., Journal of Agricultural and Food Chemistry, 58 (8) (2010), pp. 4666–4674.

Ramiro-Puig , E., Urpi-Sarda , M., Perez-Cano, F.J., Franch, A., Castellote , C., Andres-Lacueva, C., Izquierdo-Pulido, M., and Castell , M .(2007): Cocoa-Enriched Diet Enhances Antioxidant Enzyme Activity and Modulates Lymphocyte Composition in Thymus from Young Rats.J.Agric Food Chem. 8;55(16):6431-8.

Reeves, P. G.; Nielsen ,F. H and Fahey, G. C. Jr. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123:1939–1951.

SPSS (1998): Statistical Pakage for Social Science, Computer. Software, Ver. 10. SPSSCompany. London, UK.

Schermer, S. (1967): The Blood Morphologyof Laboratory Animals. p. 359. Philadelphia: F.A. Davies Co.

SPSS (1998): Statistical Pakage for Social Science, Computer. Software, Ver. 10. SPSSCompany. London, UK.

Soutar , A. K., and Naoumova , R. P., (2006) : Mechanisms of Disease: genetic causes of familial hypercholesterolemia ., Nature Clinical Practice Cardiovascular Medicine.

Varley, H.; Gewenlock, A. and Bell,M., (1980): Practical clinical biochemistry, Vol. 1, 5th ed. Pp.741:897. London; Williams HeinemenMedicalbooks, Ltd.

Velioglu, Y. S., Mazza, G., Gao, L., &Oomah, B. D., (1998): Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural and Food Chemistry, 46, 4113–4117.

Wollgast, J., Anklam, E., (2000): polyphenols in Theobromacacao: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Res Intern. 33(6):423-447.

Journal of Home Economics, Volume 26, Number (3), 2016

التأثير المحتمل لمسحوق الكاكاو على دهون الدم والحالة المضادة للأكسدة في الفئران المصابة بارتفاع كولسترول الدم

خالد عبدالرحمن شاهين , هبه عزالدين يوسف , مى محمود خفاجى و فاتن كمال راشد
قسم التغذية وعلوم الاطعمه, كلية الاقتصاد المنزلى جامعه المنوفية .مصر

الملخص العربى:

تهدف الدراسة الحالية إلى تقييم تأثير مسحوق الكاكاو على دهون الدم والحالة المضادة للأكسدة للفئران المصابة بارتفاع الكوليستيرول . حيث تم استخدام أربع وعشرون من فئران ذكور الألبينو , قسمت إلى مجموعتين رئيسيتين , المجموعة الأولى :مجموعه ضابطة سالبه (٦ فأر) , المجموعة الثانية : الفئران المصابة بالكوليستيرول (١٨ فأر) تم تقسيمها إلى ثلاث مجموعات فرعيه متساوية , المجموعة الأولى :مجموعة ضابطة موجبه، المجموعة الثانية والثالثة تناولتا الوجبة القياسية باستبدال ٢.٥ , ٥ % من الكاكاو البودر على التوالي . وقد أشارت النتائج إلى أن تناول الفئران للكاكاو أدى إلى انخفاض معنوي في مستويات الكوليستيرول ،الجليسريدات الثلاثيه ،صوره دهون الدم مقارنه بالمجموعة الضابطة الموجبة ومن ناحية أخرى وجد ان المعاملات السابقة قد أدت إلي حدوث زيادة معنوية في نشاط إنزيمات الجلوتاثيون ترانسفيريز والكتاليز مقارنة بالمجموعة الضابطة الموجبة ، لذا يمكن أن تستخلص من الدراسة إمكانية استخدام مسحوق الكاكاو ومستخلصاته في تقليل مستوي كوليستيرول الدم في الفئران المصابة بارتفاع الكوليستيرول .

الكلمات الكشافة: **كاكاو, فئران مصابة بالكوليستيرول , الفلافونات ودهون الدم .**