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Effect of Cocoa Powder and its Extracts on Lipid Profile, Oxidative Enzyme and Liver Function in Obese Rats

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Abstract: The present study aims to investigate the effects of cocoa powder *Theobroma cacao* L., cocoa water extract and cocoa ethanolic extract on lipid profile, lipoprotein, oxidative enzymes and liver functions in obese rats. Rats supplemented with cocoa powder or cocoa extracts had lower serum total lipid (TL), triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDLc), very low density lipoprotein (VLDLc), atherogenic index (AL), malondialdehyde (MDA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and glucose than obese rats. Cocoa powder or cocoa extracts supplements had higher Superoxide dismutase (SOD), glutathione peroxidase (GSH) and catalase activities than obese rats. Cocoa water extract and cocoa powder were more effective in reducing MDA than ethanolic extract. In conclusion cocoa (*Theobroma cacao* L.*)* and its extracts enhanced lipid profile, lipoprotein, oxidative enzymes and liver functions of obese rats.

Key words: Cocoa Powder · Cocoa Water Extract · Cocoa Ethanolic Extract · Lipid Profile · Phenolic Compound · Total Cholesterol (TC) · Triglycerides (TG) · Atherogenic Index (AI) · Oxidative Enzymes and Liver Functions

INTRODUCTION

The cocoa bean (*Theobroma cocoa*) has long been the main component of cocoa and chocolate [1]. Cocoa is a food source rich in polyphenols, which represent 6-8% of the dry weight of cocoa beans [2]. Cocoa is a rich source of phenolic compounds and has the highest flavanol (a polyphenol class) content of all foods on a per-weight basis [3]. Cocoa mainly contains high quantities of flavanol [4]. Cocoa and cocoa-derived products are highly consumed in many countries and because of its high content in polyphenols have recently attracted a great interest. Cocoa flavanols seem to act as highly effective chemo preventive agents against chronic diseases including cancer, heart disease, diabetes, neurodegenerative disease and ageing (reviewed in [5,6].

Numerous mechanisms have been proposed to account for the preventive effects of cocoa and its flavanols in cultured cells and animal models.

These mechanisms include the stimulation of tumor suppressor genes, induction of nitric oxide (NO) signaling and activation of the insulin pathway, among many others [7]. The antioxidant activity of cocoa polyphenols has also been suggested as potential mechanisms for cancer, CVD and diabetes prevention [8, 9].

Many studies have described cocoa phenolics as being bioactive compounds, especially prominent for their metabolic and cardiovascular effects. These effects are due, in part, to the antioxidant [10] and antiradical properties of cocoa phenolics [11], which increase the plasma level of antioxidants to prevent the oxidation of LDL-cholesterol [12]. Along with their known antiplatelet effects [13], these particular properties are related to the protective mechanism of cocoa phenolics in heart disease [12].

Obesity is one of the most common disorders in developed countries. Aside from its possible psychological and social implications, it is associated with a number of health problems like hyperlipidemia, carbohydrate intolerance, pulmonary and renal problems, pregnancy complications, hypertension, diabetics and oxidative stress [14]. Several lipid/ lipoprotein abnormalities have been observed in obese people including elevated cholesterol, triglyceride, low density

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lipoprotein (LDL) cholesterol, apolipoprotein B and lower high density lipoprotein (HDL) cholesterol levels [15]. obesity is an independent risk factor for a reduction in erythrocyte antioxidant enzyme activities and is associated with lower levels of serum antioxidants [16].

The objective of this study was to evaluate the effect of cocoa powder, cocoa water extract and cocoa ethanolic extract on lipid profile, serum lipoprotein, oxidative enzymes and liver functions in obese rats.

MATERIALS AND METHODS

Materials: Cocoa beans (*Theobrona cacao* L.) were purchased from Almarwani for spices Jeddah, Saudi Arabia.

The seeds were cleaned then dried at 40°C overnight in an electric draught oven and ground to pass through a 60 mesh sieve then kept in cold storage at 4°C for analysis.

Kits for Biochemical Analysis: Commercial diagnostic kits for estimating serum lipid profile (total cholesterol, triglycerides and lipoprotein fractions) were obtained from Randox Laboratories, U.K. The kits for estimating liver function enzymes Serum aspartate aminotransferase (AST) activity and alanine aminotransferase (ALT) activity were obtained from Diamond Company, Hannover, Germany. Antioxidant enzymes commercial kits were purchased from Roche Diagnostic laboratories, Germany.

Animals: A total number of thirty male albino rats of Wistar strain were obtained from the experimental Animal Unit of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia.

Preparation of the Basal Diet: The basal diet for rats was prepared using AIN-93 according to Reeves *et al.* [17]. The basal diet consists of the following: Protein (Casein) 20%; Sucrose 10%; Corn Oil 4%; Choline chloride 0.2%; Vitamin mixture 1%; Salt mixture 3.5%; Fibers (Cellulose) 5% and the remainder is Corn Starch up to 100%.

Induction of Obesity: Induction of obesity was induced by feeding the rats on basal diets supplemented with 10% animal lipids.

Experimental Design of Rats: The experiment was performed on thirty male mature Wistar rats. Animals were distributed randomly into five equal groups, six rats each. Rats were housed in standard plastic cages at a room temperature (24± 2 °C), with fixed 12-hour lighting system. All rats were allowed to free access to basal diet and water for one week before starting the experiment for acclimatization. After acclimatization period, the rats were allocated in to the following groups:

Group 1 (n= 6): Rats were fed on the basal diet only, kept as a negative control group. *Group 2 (n= 6)*: Rats were fed on the basal diet supplemented with 10% animal lipids, kept as a positive control group. *Group 3 (n= 6)*: Obesity rats were fed on basal diet supplemented with 10% cocoa powder of the weight of rats. *Group 4 (n= 6)*: Obesity rats were fed on basal diet and administrated given orally by gavage cocoa water extract. One g /1kg body weight / day for 6 weeks of the weight of rats. *Group 5(n= 6)*: Obesity rats were fed on basal diet and administrated given orally by gavage cocoa ethanolic extract. 1 One g /1kg body weight / day for 6 weeks of the weight of rats. At the end of the experimental period, all rats were fasted overnight then sacrificed. Blood samples were immediately collected from the retro orbital plexus with capillary tubes under mild ether anesthesia, into clean dried centrifuge tubes. The tubes were then centrifuged at 3000 rpm for 15 minutes. Clear serum samples were carefully separated using Pasteur pipettes and frozen at - 20°C until biochemical analysis [18].

Preparation of Extracts: Water extract was prepared according to the method of Veliglu *et al.* [19], the dried cocoa powder (100g) was soaking in 500 ml distilled water for 2 h. at 50° C by orbital shaker. The extract was filter and dried using freeze drying system under reduced pressure.

Ethanolic extract was made by soaking in dried cocoa powder (100g) were extracted in 1000 ml of 70% aqueous ethanol for 3 days. The extract was filter and ethanol was evaporated under reduced pressure at 50° C using a rotary evaporator. The remaining water extract was dried using freeze drying system under reduced pressure.

The dried water and ethanolic extracts were dissolved in distilled water to a concentration of 1g/ml before administration in obese rats.

Biochemical Analysis: Total Phenolic Content in cocoa powder were determined and identified by High-performance liquid chromatography (HPLC) according to the method reported by Mattila *et al*. [20].

Antioxidant Activity: Radical Scavenging Activity (RSA %) assay Free radical Scavenging activity (RSA) of the samples was measured using the method of Brand-Williams *et al.* [21].

Serum Analysis: Serum cholesterol (TC) was determined according to the method described by Allain *et al*. [22],

Concentrations of serum triglycerides (TG) were determined according to the method described by Trinder [23].

Serum high density lipoprotein cholesterol (HDL-c) was calorimetrically determined according to the method described by Lopes-Virella *et al.* [24].

Serum low density lipoproteins cholesterol (LDL-c) was calorimetrically determined according to the method described by Friedewald *et al.* [25].

Serum very low density lipoproteins cholesterol (VLDL-c) was calorimetrically determined according to the method described by Friedewald *et al.* [25].

Determination of Serum Aspartate Aminotransferase (AST) Activity and Alanine Aminotransferase (ALT) Activity: Serum aspartate aminotransferase (AST) activity and alanine aminotransferase (ALT) activity were estimated enzymatically based on color reaction formation. The developed color was measured according to the method described by Bergmeyer *et al.* [26]. The frozen liver samples were thoroughly homogenized on ice with Tri- HCL buffer solution (PH 7.4) to obtain 10 % tissue homogenate. The prepared liver homogenates were used for measurement of activities of antioxidant enzymes [27].

Catalase activity was measured by monitoring the decomposition of H_2O_2 at 240 nm wave length (extinction coefficient 0.00394 ± 0.0002 mM^{-1} mm^{-1}) according to the method described by Sinha [28]. CAT enzyme activity was expressed as U of catalase/mg protein (1 unit of catalase is defined as the amount of enzyme required to hydrolyze 1 μmol of H_2O_2 per min).

Superoxide dismutase (SOD) activity was assessed using a Xanthine oxidase system to generate superoxide radicals (O_2^-) as described by Kakkar *et al.* [29]. The rate of suppression of the reduction of Nitro tetrazolium blue (NTB) by O_2^- was monitored at 550 nm wave length. SOD enzyme activity was expressed as U of SOD/mg of protein (1 unit of SOD is defined as the amount of enzyme required to inhibit the rate of reduction of NTB by 50%.

Glutathione peroxidase (GSH) activity was assayed by NADPH oxidation at 340 nm wave length when GSSG is reduced back by glutathione reductase as described by Paglia and Valentine [30], 1 unit of (GSH) is defined as the amount of enzyme required to convert 1 nmol NADPH to $NADP^+$ per min).

The Atherogenic index was determined according to the method described by Mertz [31].

Serum glucose was measured by enzymatic GOD / POD kits according to the method by Trinder [23].

The method is based on the reaction of thiobarbituric acid with lipid peroxides malondialdehyde (MDA) in acidic medium at 95°C for 45 minutes to form thiobarbituric acid was determined as described by Sedlak and Lindsay [32].

Statistical Analysis: Statistical analysis was done by using (SPSS) Statistical Package for the Social Sciences for Windows, version 22 (SPSS Inc., Chicago, IL, USA). Collected data was presented as mean ± standard error (SE). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to Armitage and Berry [33]. All differences were consider significant if P □ 0.05.

RESULTS AND DISCUSSION

The total phenolic compound of cocoa powder was 590 mg gallic acid /100 g dry samples.

Identification of phenolic compound of cocoa powder was presented in Fig (1). The phenolic compounds of cocoa powder were O- coumaric, gallic, phenol, p-Oh-benzoic, chlorogenic, prolocatechuric. O- coumaric content was the highest 8.98% phenolic compounds in cocoa powder, while chlorogenic acid was the lowest 0.59% phenolic compounds in cocoa powder. These results are agreement with Grassi *et al.* and Vinson, Proch and Zubik [2, 3].

The proximate antioxidant activity and total phenolic compounds of cocoa water extract and cocoa ethanolic extracts are presented in Table (1). Data showed that the antioxidant activity and total phenolic compounds in cocoa water extract significant higher ($P<0.05$) than cocoa ethanolic extract. Water is more efficient to extract phenolic compounds than ethanol and consequently antioxidant activity for cocoa water extract was significant higher than ethanolic extract. These results are agreement with Vinson [3].

The effects of cocoa powder, cocoa water extract and cocoa ethanolic extract on body weight performance of normal and obese rats are presented in Table (2). At the beginning of the experiment, there was no significant difference in initial weight between positive control and other groups (cocoa treated groups). at the end of the experiment, the relative body weight gain was significantly reduced by - 13.23% by treatment of cocoa powder, followed by cocoa ethanolic extract and cocoa water extract are -10.17% and - 7.66% respectively, while the positive control was increased by 48.43%. Lecumberri *et al.* [34] reported that the cocoa ingestion produces its ant obesity effects by affecting lipid metabolism.

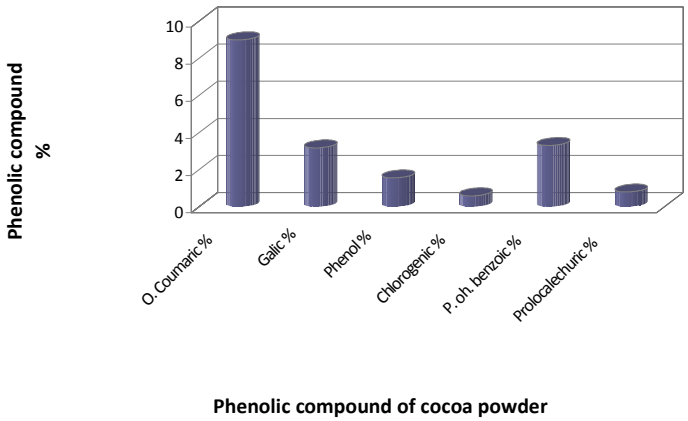

Fig. 1: Phenolic compounds of cocoa powder

Table 1: Proximate antioxidant activity and total phenolic compound of cocoa extracts

Type of cocoa	Antioxidant activity (%)	Total phenolic mg gallic/ ml
Cocoa ethanolic extract	$57.97^{b} \pm 3.37$	$349.09^{b} \pm 6.13$
Cocoa water extract	$66.78^{a} \pm 1.47$	$483.38^{a} \pm 5.93$

Means in the same column with different letter are significantly different P<0.05

Table 2: Effect of cocoa powder and cocoa extracts on body weight performance of normal and obese rats

	Groups					
		Obese Groups				
Variable	Negative Control (Normal)	Positive Control	Cocoa Powder	Cocoa ethanol extract	Cocoa water extract	L S D
Initial weight (g)	$122.8^{b} \pm 2.79$	$209.0^{a} \pm 2.76$	$207.67^{a} \pm 2.73$	$2011.0^{a} \pm 2.89$	$209.84^{a} \pm 3.19$	3.42
Final Weight (g)	$132.33^{d} \pm 1.86$	$310.17^{a} \pm 2.93$	$180.17^{c} \pm 2.23$	$189.50^{b} \pm 2.35$	$190.84^{b} \pm 3.19$	3.04
Relative Gain (%)	7.77 ± 2.21	48.43 ± 3.34	-13.23 ± 1.14	-10.17 ± 1.84	-7.66 ± 3.82	

Means in the same raw with different letter are significantly different P<0.05.

Table 3. Effect of cocoa powder and cocoa extracts on serum lipid profile of normal and obese rats

		Obese Groups				
Parameters (mg/dl)	Negative Control (Normal)	Positive Control	Cocoa Powder	Cocoa ethanol extract	Cocoa water extract	L S D
Total lipids	$230.69^{e} \pm 2.79$	$384.71^{a} \pm 2.82$	$280.04^{b} \pm 2.19$	$263.39^{c} \pm 2.36$	$248.61^{d} \pm 2.59$	3.38
Triglyceride	$42.86^{d} \pm 2.04$	$64.76^{a} \pm 2.11$	$53.40^{b} \pm 2.09$	$56.04^{b} \pm 1.79$	$49.75^{c} \pm 0.89$	2.77
Total cholesterols	$88.79^{e} \pm 2.54$	$157.38^{a} \pm 4.23$	$119.83^{c} \pm 2.95$	$129..18^{b} \pm 2.53$	$100.78^{d} \pm 2.33$	3.95

Means in the same raw with different letter are significantly different P<0.05.

The Effect of cocoa powder and cocoa extracts on serum lipid profile of normal and obese rats is shown in Table (3). Obese rats had higher significantly different P<0.05 TL, TG and TC than normal rats. The serum TL, TG and TC in obese rats supplemented with cocoa powder or cocoa extracts were significantly (P<0.05) lower than positive control rats. Water extract was more effective in reducing serum TL, TG and TC than cocoa powder and ethanolic extract. Ethanolic extract was more effective (P<0.05) in reducing TL than cocoa powder. However, TC had an opposite trend. On the other hand, no significant (P<0.05) differences in TG was observed between rats supplemented with cocoa powder and rats supplemented with ethanolic extract. Lecumberri *et al.* [35], reported that the consumption of cocoa fiber with hypercholesterolemia diet improved the lipid profile.

Effect of cocoa powder and cocoa extracts on serum lipoprotein and atherogenic index of normal and obese rats are shown in Table (4). Negative control rats had lower (P<0.05) LDLc, VLDKc and AI values than obese rats. However, HDLc value in negative control rats had an opposite effect. Positive control rats had higher (P<0.05) LDLc, VLDLc and AI values than rats supplemented with cocoa powder or cocoa extracts, while, positive control

Table 4. Effect of cocoa powder and cocoa extracts on serum lipoprotein and atherogenic index of normal and obese rats

		Obese Groups				
Parameters (mg/dl)	Negative Control (Normal)	Positive Control	Cocoa Powder	Cocoa ethanol extract	Cocoa water extract	L S D
HDLc	$51.64^{a} \pm 1.55$	$37.09^{d} \pm 2.15$	$44.07^{b} \pm 1.06$	$41.13^{c} \pm 0.69$	$45.48^{b} \pm 1.60$	1.98
LDLc	$27.34^{e} \pm 1.58$	$107.34^{a} \pm 4.01$	$64.49^{c} \pm 3.81$	$76.66^{b} \pm 2.49$	$45.29^{d} \pm 2.12$	3.90
VLDLc	$8.45^{d} \pm 0.35$	$12.95^{a} \pm 0.62$	$10.68^{b} \pm 0.42$	$11.21^{b} \pm 0.35$	$9.95^{c} \pm 0.17$	0.54
AI	$0.713^{e} \pm 0.04$	$3.25^{a} \pm 0.24$	$1.68^{c} \pm 0.11$	$2.14^{b} \pm 0.06$	$1.23^{d} \pm 0.07$	0.17

Means in the same raw with different letter are significantly different P<0.05.

Table 5: Effect of cocoa powder and cocoa extracts on serum glucose level of normal and obese rats

		Obese Groups				
Parameters (mg/dl)	Negative Control (Normal)	Positive Control	Cocoa Powder	Cocoa ethanol extract	Cocoa water extract	L S D
Glucose	$106.64^{e} \pm 1.53$	$143.72^{a} \pm 2.29$	$129.54^{c} \pm 1.91$	$134.60^{b} \pm 2.04$	$124.71^{d} \pm 2.05$	2.6

Means in the same raw with different letter are significantly different P<0.05.

Table 6: Effect of cocoa powder and cocoa extracts on oxidative enzyme of normal and obese rats

		Obese Groups				
Parameters	Negative Control (Normal)	Positive Control	Cocoa Powder	Cocoa ethanol extract	Cocoa water extract	L S D
MDA nmol/ml	$2.16^{d} \pm 0.16$	$3.04^{a} \pm 0.18$	$2.50^{bc} \pm 0.10$	$2.62^{b} \pm 0.08$	$2.36^{c} \pm 0.12$	0.118
SOD unit /prol.	$31.96^{a} \pm 1.57$	$13.56^{d} \pm 1.11$	$21.08^{c} \pm 1.60$	$20.16^{c} \pm 1.08$	$24.71^{b} \pm 1.05$	1.756
GSH mg/dl	$144.92^{a} \pm 3.54$	$101.54^{d} \pm 2.88$	$117.92^{c} \pm 2.06$	$116.0^{c} \pm 2.22$	$130.62^{b} \pm 2.14$	3.47
Catalase	$804.70^{a} \pm 4.42$	$594.24^{e} \pm 3.58$	$675.68^{c} \pm 3.45$	$613.36^{d} \pm 3.77$	$756.34^{b} \pm 4.47$	5.48

Means in the same raw with different letter are significantly different P<0.05.

rats had a lower (P<0.05) HDLc values than rats supplemented with cocoa powder or cocoa extracts. Baba *et al*. [36], found that, cocoa flavonols were increased the concentration of HDL. Water extract was more effective P<0.05 in reducing LDLc, VLDLc and AI values than cocoa powder or ethanolic extract. Cocoa powder was more effective (P<0.05) in reducing LDLc and AI than ethanolic extract. No significant (P<0.05) differences was found in VLDLc value between cocoa powder and ethanolic extract. These results are in agreement with those obtained [36, 37], suggested that the regular consumption of cocoa products containing flavonols may reduce risk of cardiovascular disease (CVD). Kurosawa *et al.* [38] consider that antioxidative activity of polyphenol rich in cocoa powder may be a key factor for the anti-atherosclerotic effect.

The effect of cocoa powder and cocoa extracts on serum glucose level of normal and obese rats are presented in Table (5). Obese rats had a higher (P<0.05) serum glucose than negative control rats. Rats supplemented with cocoa powder or cocoa extracts had lowered (P<0.05) serum glucose than positive control rats. Water extract and cocoa powder were more (P<0.05) effective in reducing serum glucose than ethanolic extract. Similar results were obtained by Jalil *et al.* [39], who found that cocoa supplementation in obese rats are reduced plasma glucose compared to unsupplemented obese diabetic rats.

The effects of cocoa powder and cocoa extracts on oxidative enzyme of normal and obese rats are illustrated in Table (6). Obese rats had a higher (P<0.05) MDA than negative control rats. Rats supplemented with cocoa powder and cocoa extracts had a lower (P<0.05) MDA than positive control rats. Water extract and cocoa powder was more (P<0.05) effective in reducing MDA than ethanolic extract. Many studies have shown that levels of biomarkers of lipid peroxidation such as thiobarbituric acid-reactive substances were not modified as a consequence of cocoa consumption by healthy individuals [40, 41], found that cacao liquor polyphenols intake resulted in a decrease in oxidative stress. Positive control rats had lower (P<0.05) SOD, GSH and catalase activities than rats supplemented with cocoa powder or cocoa extracts. Rats supplemented with cocoa water extract had higher (P<0.05) SOD, GSH and catalase activities than rats supplemented with cocoa powder and cocoa ethanolic extract. There were no significant (P<0.05) differences in SOD and GSH between rats supplemented with cocoa powder and cocoa ethanolic extract. Ramiro *et al.* [43] found that cocoa diet enhances thymus antioxidant defenses and influences thymocyle differentiation. Cocoa supplementation in obese diabetic rats may enhance the antioxidant defense system [39, 42], found that cacao liquor polyphenols intake resulted in a decrease in oxidative stress without maintaining vitamin E in the plasma and the tissues.

Table 7: Effect of cocoa powder and cocoa extracts on liver function-of normal and obese rats

		Obese Groups				
Parameters IU/l	Negative Control (Normal)	Positive Control	Cocoa Powder	Cocoa ethanol extract	Cocoa water extract	L S D
ALT	$83.99^{c} \pm 2.33$	$103.62^{a} \pm 1.78$	$92.09^{b} \pm 1.65$	$92.01^{b} \pm 0.85$	$90.26^{b} \pm 1.98$	2.38
AST	$45.90^{c} \pm 1.60$	$52.71^{a} \pm 2.89$	$49.25^{b} \pm 1.78$	$48.55^{b} \pm 1.25$	$49.79^{b} \pm 1.21$	2.43
ALP	$123.67^{c} \pm 2.60$	$136.17^{a} \pm 1.95$	$127.81^{b} \pm 1.19$	$128.72^{b} \pm 1.51$	$127.08^{b} \pm 1.39$	2.31

Means in the same raw with different letter are significantly different $P<0.05$.

The effects of cocoa powder and cocoa extracts on liver function of normal and obese rats are presented in Table (7). Positive control rats has higher ($P<0.05$) ALT, AST and ALP activities than rats supplemented with cocoa powder and cocoa extracts. No significant ($P<0.05$) differences in ALT, AST and ALP activities were observed between rats supplemented with cocoa powder and rats supplemented with cocoa extracts. These results are agreement with Martín *et al.* [6].

From the above results, it could be concluded that, the cocoa powder or cocoa extracts enhanced lipid profile, lipoprotein, oxidative enzymes and liver functions of obese rats.

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