



EFFECT OF DIETARY SUPPLEMENTATION WITH *CYPHOSTEMMA DIGITATUM* ON SERUM LIPID PROFILE AND LIVER ENZYMES IN HYPERLIPIDEMIC SUBJECTS

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ABSTRACT

Aims: To examine the effects of the administration of CD on lipid metabolism and liver enzymes in hyperlipidemic subjects. Study design: 20 subjects were divided into two groups, each containing ten subjects. Each subject in the 1st group ingested 1g, while the subject in the 2nd group ingested 2g of CD powder as a broth suspension in a single daily dose before lunch during the follow up period of one month. Patients kept their individual diet relatively constant. Methodology: We included 20 hyperlipidemic volunteers (men; age range 20-50 years) of a total of 80 subjects. Blood samples were taken and analyzed for total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), atherogenic index (AI), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Results: The results of the present study show a significant decrease in the levels of TC, LDL-C and TG in 1g and 2g of CD administration groups by 12.6, 24.1, 17.1% and 19.9, 25.5 23.4% respectively while the level of HDL-C was significantly increased by 45.6 and 32.5% in 1g and 2g respectively. The atherogenic index (AI) was significantly decreased in 1g and 2g groups by and 47.4% and 45.5% respectively. Our results also show that, CD administration in 1g and 2g subjects causes a significant decrease in ALT and AST by 39.2, 32% and 48.5, 33.3% respectively while the level of ALP was non-significantly decreased. Conclusion: dietary supplementation with CD administration decreased serum lipid profile and liver enzymes in hyperlipidemic subjects. Therefore, it may be regarded as a useful therapy for hyperlipidemia and liver disease. However, further studies are required

to compare it with other medicinal plants and with lipid lowering drugs. Place and Duration of Study: Department of Chemistry, Ibb University, Yemen, between February to June 2012.

Keywords: *Cyphostemma digitatum*, Lipid profile, Male subjects, Yemen.

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Contribution/ Originality

This study is one of very few studies which have investigated the Effect of Dietary Supplementation with *Cyphostemma Digitatum* on Serum Lipid Profile and Liver Enzymes in Hyperlipidemic Subjects (human) and we found that, dietary supplementation with CD administration decreased serum lipid profile and liver enzymes in hyperlipidemic subjects.

1. INTRODUCTION

Cardiovascular disease (CVD) is believed to become the most leading cause of morbidity and mortality in men and women in the world in 2020 [1-3] and two thirds of all cardiovascular fatalities occur in developing countries [4, 5]. Hyperlipidemia (mainly increased level of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) along with decrease in high-density lipoprotein cholesterol (HDL-C) is the predictor of CVD [6]. Hyperlipidemia has an indirect role by stimulating the production of oxygen free radicals [7]. According to global estimates from WHO, nearly 1 of every 3 deaths in 2004 was attributed to cardiovascular disease with nearly 80% of these deaths occurring in low- and middle-income Countries [8]. The search for new drugs able to reduce and/or to regulate serum cholesterol and triacylglycerol levels has gained importance over the years, resulting in numerous reports on significant activities of natural agents [9]. There is resurgence in the use of herbal medicines worldwide. An estimated one third of adults in the Western world use alternative therapies, including herbs. These herbs may be used either in their primary forms or combined into mixtures. In contrast to chemical drugs, herbs have sometimes been claimed to be non-toxic, because of their natural origin and long-term use as folk medicines [10]. Although plant extracts constitute potential candidates, they often contain complex mixture of many different compounds with distinct polarity, antioxidant and pro-oxidant properties [11, 12]. *Cyphostemma digitatum* (CD) (Vitaceae), (locally known as "Halka" in Yemen), is one of the most medicinal and culinary herbs used with high demand in Yemen. It is a perennial, climbing, and succulent under shrub with compound fleshy leaves and tendrils [13]. The leaves and fleshy young stem branches are used in dried form after processing. Usually dry food discs (8–12 cm in diameter with irregular 1–5 mm thickness) are commercially produced which are regularly used to prepare many traditional dishes or as a source for food flavoring. It has been also used in traditional medicine for vomiting, against malaria and headache and for general health support [14]. In addition, it was reported that CD contains high amounts of vitamin C, vitamin A, vitamin E, and β -carotene [14]. To date, the hypolipidemic effects of CD have never been reported. In our previous work, the hepatoprotective effects of the aqueous leaves extract of CD against CCl₄ -induced acute liver

injury in guinea pigs is recently documented [15]. The present study is aimed to investigate whether administration of CD in humans would alter serum lipid levels and/or have hepatoprotective effects.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1. Plant Material

Fresh leaves of CD were harvested from nature in Aug- Sep, 2011 in Ba'dan countryside in the southwest highlands in central Yemen. The plant was authenticated by comparison with reference specimens preserved at the Herbarium of Biological Department, Ibb University. The clean leaves were boiled for 30 min under pressure, then the water was removed and the leave mass was mixed with wood spoon. The thick homogeneous stature baste was thinned into disks (8– 12 cm in diameter with irregular 1– 5 mm thickness) and dried in the sun in clean plates covered with tiny mesh, changing upside down each day until complete dryness [13], after that the dry disks were blending by a mixer to powder form.

2.2. Study Subjects

Healthy volunteers were recruited from the local community in Ibb, Yemen through an advertisement. The proposed criteria were healthy males aged between 20 and 50 y. They were interviewed for details of their age, weight, height, smoking, and Qat chewing. Body mass index (BMI) was calculated for each subject. Subjects with hypertension, coronary, peripheral or cerebral vascular disease were excluded from participating in the study. This study was carried out between Feb to Jun 2012 at the Department of Chemistry, Ibb University, Yemen. All subjects gave written informed consent to participate. The study was approved by the Local Ethics Committee. Fasting blood samples were taken from all above subjects and analyzed for total cholesterol (TC), triglycerides (TG), and high density lipoprotein cholesterol (HDL-C) levels. Low density lipoprotein cholesterol (LDL-C) levels were calculated. Only hyperlipidemic subjects (20 subjects) were selected to the 2nd step of this work. Hyperlipidemic subject is a subject who had $TC \geq 4.5$ mmol/ L (175 mg/dl) and/ or $TG \geq 1.7$ mmol/ L (150 mg/dl) [16, 17].

2.3. Experimental Design

The 20 subjects were divided into two groups, each containing ten subjects. Each subject in the 1st group ingested 1g, while the subject in the 2nd group ingested 2g of CD powder as a broth suspension in a single daily dose before lunch during the follow up period of one month. Patients kept their individual diet relatively constant. No subject had any complaint during the period of the plant powder administration. No concomitant medication intended to influence serum lipid level was permitted.

2.4. Blood Sampling and Analysis

Fasting blood samples were collected from all subjects before and after the one month CD administration as control and treated sampling. Serum samples were obtained and frozen in dry ice prior to being stored at -80°C . The separated serum samples were used for estimation of TC [18], TG [19], and HDL-C [20], using Spinreact kits (Spain). Low density lipoprotein cholesterol (LDL-C) levels were calculated using Friedwald equation [21]. The atherogenic index (AI) was calculated by using the formula, $\text{TC-HDL-C}/\text{HDL-C}$ [22]. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the method of Tietz [23] using Spinreact kits (Spain). Alkaline phosphatase (ALP) was determined according to the method of King [24] using Spinreact kits (Spain). The enzyme activity was expressed as U/l.

2.5. Statistical Analysis

For statistical analysis the SPSS computer program was used. The statistical analysis was carried out by one-way ANOVA setting the probability level to $P<.05$, post hoc analysis of group differences was performed by LSD to test the differences in mean values of each variable between before and after one month of CD administration.

3. RESULTS AND DISCUSSION

The basal characteristics of the study groups are shown in (Table 1). There were no significant differences between subjects who were treated daily with 1g or 2g CD for age, sex, and BMI. All subjects completed the treatment period without any observed side effects. In both groups, there were two smoking subjects (20%), while the qat chewing in 1g and 2g/day groups were 4 and 8 subjects respectively.

Table-1. Demographic information and body measurements of the study subjects

Parameter	1g CD group	2g CD group
Age (years)	30.1 ± 8.4	33.1 ± 7.9
Height (cm)	167.7 ± 10.8	169.8 ± 5.1
Weight (kg)	71.8 ± 13.4	77.7 ± 9.4
BMI (kg/m^2)	25.2 ± 2.7	26.8 ± 3.1
Smoking (%)	20%	20%
Qat chewing (%)	40%	80%

Values are presented as $\bar{X} \pm \text{S.D}$, and the number of subjects was $N=10$ for each group. There was a significant ($P<0.05$) decrease in the levels of TC, LDL-C and TG in 1g group of CDP administration by 12.6%, 24.1% and 17.1% respectively while the level of HDL-C was significantly ($P<0.05$) increased by 45.6% after one month of treatment. In 2g subjects of CDP administration the levels of TC, LDL-C and TG (Table 2) were also significantly ($P<0.05$) decreased by 19.9%, 25.5 and 23.4% respectively while the level of HDL-C was significantly ($P<0.05$) increased in this group by 32.5%. The Atherogenic index (AI) was significantly decreased in 1g and 2g groups by 47.4% and 45.5% respectively (Table 2). The elevated

atherogenic index, i.e. TC/HDL ratio, is a useful determinant of cardiovascular risk [25]. Our results showed that CD administration by 1g and 2g daily for one month to hyperlipidemic subjects had a strong hypotriglyceridemic and hypocholesterolemic effects with a reduction of plasma LDL-C levels and an increase in HDL-C levels. These results were in agreement with our previous work on animals [15].

Table-2. Changes in serum TC, LDL-C, HDL-C, TG and AI before and after one month of 1g and 2g of CD administration

Parameter	1g group		2g group	
	Baseline	CD	Baseline	CD
TC (mg/dl)	185.7 ± 26.4	162.3 ± 25.2*	190.6 ± 44.7	152.7 ± 20.6*
LDL-C (mg/dl)	100.0 ± 18.1	75.9 ± 16.7*	94.8 ± 28.4	70.6 ± 14.0*
HDL-C (mg/dl)	36.4 ± 6.3	53.0 ± 8.2*	41.2 ± 10.9	54.6 ± 11.6*
TG (mg/dl)	195.3 ± 34.4	161.9 ± 31.1*	216 ± 35.8	165.5 ± 31.8*
AI	4.3 ± 1.9	2.2 ± 1.2*	3.96 ± 1.2	2.1 ± 0.8*

Each value represents the $\bar{X} \pm S.D.$, N=10. Values marked with asterisks differ significantly $P < 0.05$ from baseline.

The major role of hyperlipidemia in the pathogenesis of atherosclerosis has been implicated [26, 27]. The mechanism of hypolipidemic action is not fully understood; however it may be related to decreased cholesterol synthesis, decreased dietary cholesterol absorption, and increased cholesterol excretion through bile acid formation [28]. Serum TG and HDL-C have been observed to be inversely related [29]. Our results show that 20% of the subjects had moderate hypertriglyceridaemia. It is well known that hypertriglyceridaemia is a significant independent CVD risk factor [30] but it seems that the association is not as strong as for hypercholesterolemia [31]. The hypotriglyceridemic effects of CD administration in both 1g and 2g groups may be through CD effect upon increasing the activity of lipase [32] and/or by the inhibition of hepatic lipogenesis [33]. Moreover it has been reported that, β -carotene reduced the elevation of cholesterol and triglycerides of diabetic rats [34]. In addition, it has been reported that every 1.0 mmol/L reduction in LDL cholesterol is associated with a corresponding 20–25% reduction in CVD mortality and non-fatal myocardial infarction [17]. The increasing of HDL-C in this study suggested that CD could have protective effect on cardiovascular system because numerous studies have demonstrated that high levels of HDL-C are associated with a lower incidence of CVD [35, 36] or might be due to stimulation of pre- β HDL-C and reverse cholesterol transport as demonstrated by previous findings [37, 38]. Moreover, epidemiological studies have shown that high HDL-C levels could potentially contribute to its anti-atherogenic properties, including its capacity to inhibit LDL oxidation and protect endothelial cells from the cytotoxic effects of oxidized LDL [39]. The hypolipidemic effect of CD does not seem to be due only to one component, but rather to the synergistic action of its different constituents, including vitamins (C, E and A) soluble fiber (e.g. mucilage), sterols and flavonoids. It is well-known that vitamin C, vitamin E, and β -carotene all displayed antioxidant activity and thus provided a cellular defense against reactive oxygen species, which could damage the DNA [40, 41]. The role of these vitamins is emphasized by their inhibitory action on the oxidative modification of LDL

[42] and their improvement of endothelial dysfunction [43, 44]. Vitamin E is the main chain-breaking lipid-soluble vitamin in plasma and LDL [45]. Vitamin E can potentially reduce the progression of atherosclerosis by reducing adhesion molecule expression [46] and by enhancing NO bioavailability [47]. Vitamin C has also been shown to enhance NO synthesis in endothelial cells and it has been shown to have sustained beneficial effects on endothelial-derived NO-dependent in vivo [48]. Moreover, it has been reported that vitamin C has measurable effects on LDL oxidation indices and might be beneficial in prevention damages resulted in atherosclerosis [49]. Supplementation of diets with α -tocopherol significantly increased plasma α -tocopherol levels and resulted in lesser accumulation of peroxides in plasma [50, 51]. In addition, previous reports indicated that serum carotenoid levels have been inversely associated with risk of atherosclerosis [52], atherogenic factors [53] and cardiovascular mortality [54]. The pro-oxidant effects of β -carotene have been proposed to be due to the tendency of β -carotene radicals reacting with oxygen to give rise to peroxy radicals that mediate lipid peroxidation [55]. In fact the combination of vitamins C and E exhibited a stronger positive effect than vitamin C or vitamin E did on their own. It was reported that cooperative interactions between vitamins C and E had protecting effects against lipid peroxidation in liposomes [56] and pesticide intoxication [57]. It was also reported that the role of vitamin C is to prevent the consumption of hydrophobic antioxidant vitamins such as vitamin E and β -carotene [58] and ensure their recycling [59], therefore playing an important role in maintaining antioxidative protection. Our results also show that CD administration in 1g and 2g subjects for one month causes a significant ($P < .05$) decreasing of ALT and AST by 39.2%, 32% and 48.5%, 33.3% respectively while the level of ALP was non-significantly ($P > .05$) decreased (Table 3). This effect was in accordance with our previous report on animals where we concluded that CD extract caused a hepatoprotective effect and induced restoration of hepatic enzymes (AST, ALT and ALP) in CCl₄-intoxicated guinea pigs [15]. The mechanism of hepatoprotection of CD could be explained through an inhibition of activity of cytochrome P450 enzymes as mentioned by Zhao, et al. [60]. Other mechanism may be due to the antioxidant activity of CD because of its high total phenolic compound and vitamins content as reported by [13, 14].

Table-3. Changes in serum ALT, AST, and ALP before and after one month of 1g and 2g of CD administration.

Parameter	1g group		2g group	
	Baseline	CD	Baseline	CD
ALT	5.1 \pm 1.4	3.1 \pm 0.9*	3.3 \pm 1.3	1.7 \pm 0.7*
AST	5.0 \pm 1.0	3.4 \pm 1.0*	3.0 \pm 1.2	2.0 \pm 0.8*
ALP	10.2 \pm 1.6	8.3 \pm 1.6*	14.7 \pm 5.7	11.8 \pm 4.0*

Each value represents the $\bar{X} \pm S.D.$, N=10. Values marked with asterisks differ significantly $P < 0.05$ from baseline.

4. CONCLUSION

In conclusion, dietary supplementation with CD administration decreased serum lipid profile and liver enzymes in hyperlipidemic subjects. Therefore, it may be regarded as a useful therapy

for hyperlipidemia and liver disease. However, further studies are required to compare it with other medicinal plants and with lipid lowering drugs.

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