

This Provisional PDF corresponds to the article as it appeared upon acceptance. Copyedited and fully formatted PDF and full text (HTML) versions will be made available soon.

The effect of *cissus quadrangularis* (CQR-300) and *Cissus* formulation (CORE) on obesity and obesity-induced oxidative stress

Lipids in Health and Disease 2007, **6**:4 doi:10.1186/1476-511X-6-4

Julius E Oben (juliusoben@hotmail.com)

Damaris Mandob Enyegue (damand04@yahoo.fr)

Gilles I Fomekong (fdogines@yahoo.fr)

Yves B Soukoutoua (soukyberto@yahoo.fr)

Gabriel A Agbor (agoga@yahoo.fr)

ISSN 1476-511X

Article type Research

Submission date 15 January 2007

Acceptance date 4 February 2007

Publication date 4 February 2007

Article URL <http://www.lipidworld.com/content/6/1/4>

This peer-reviewed article was published immediately upon acceptance. It can be downloaded, printed and distributed freely for any purposes (see copyright notice below).

Articles in *Lipids in Health and Disease* are listed in PubMed and archived at PubMed Central.

For information about publishing your research in *Lipids in Health and Disease* or any BioMed Central journal, go to

<http://www.lipidworld.com/info/instructions/>

For information about other BioMed Central publications go to

<http://www.biomedcentral.com/>

The effect of *Cissus quadrangularis* (CQR-300) and a *Cissus* formulation (CORE) on obesity and obesity-induced oxidative stress

Julius E. Oben¹*, Damaris Mandob Enyegue¹, Gilles I. Fomekong¹, Yves B. Soukontoua, and Gabriel A. Agbor^{1,2}

1. *Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, B.P. 812, University of Yaoundé I, Yaoundé, Cameroon.*
2. CRPMT, Institute of Medical Research and Medicinal Plants Studies, Yaoundé, Cameroon

*Corresponding Author: Professor Julius Oben,
Laboratory of Nutrition and Nutritional Biochemistry
Department of Biochemistry
BP 812,
University of Yaounde I
Yaounde
Cameroon

Email: juliusoben@hotmail.com

Abstract

Aim: Obesity is generally linked to complications in lipid metabolism and oxidative stress. The aim of this study was to compare the effect of a proprietary extract of *Cissus quadrangularis* (CQR-300) to that of a proprietary formulation containing CQR-300 (CORE) on weight, blood lipids, and oxidative stress in overweight and obese people.

Methods: The first part of the study investigated the *in vitro* antioxidant properties of CQR-300 and CORE using 3 different methods, while the second part of the study was a double-blind placebo controlled design, involving initially 168 overweight and obese persons (38.7% males; 61.3% females; ages 19-54), of whom 153 completed the study. All participants received two daily doses of CQR-300, CORE, or placebo and were encouraged to maintain their normal levels of physical activity. Anthropometric measurements and blood sampling were done at the beginning and end of the study period.

Results: CQR-300 as well as CORE exhibited antioxidant properties *in vitro*. They also acted as *in vivo* antioxidants, bringing about significant ($p<0.001$) reductions in plasma TBARS and carbonyls. Both CQR-300 and CORE also brought about significant reductions in weight, body fat, total cholesterol, LDL-cholesterol, triglycerides, and fasting blood glucose levels over the respective study periods. These changes were accompanied by a significant increase in HDL-cholesterol levels, plasma 5-HT, and creatinine.

Conclusion: CQR-300 (300 mg daily) and CORE (1028 mg daily) brought about significant reductions in weight and blood glucose levels, while decreasing serum lipids thus improving cardiovascular risk factors. The increase in plasma 5-HT and creatinine for both groups hypothesizes a mechanism of controlling appetite and promoting the increase of lean muscle mass by *Cissus quadrangularis*, thereby supporting the clinical data for weight loss and improving cardiovascular health.

Background:

The incidence of obesity in adults as well as children is on an increase globally. Once considered a problem of developed countries, this global epidemic also affects developing countries. Coupled to this epidemic are obesity-related complications such as cardiovascular disease, stroke, depression, and Type-2 diabetes, which are spreading rapidly across poor and middle-income countries, where infectious diseases and malnutrition have previously overshadowed such illnesses [1]. Obesity is a principal causative factor of metabolic syndrome [2,3,4,5, 6]. The coexistence of these metabolic syndromes (hyperglycemia, dyslipidemia, and hypertension in the same individual) is a growing medical problem in industrialized countries [6,7,8,9]. It has been reported that obesity may induce systemic oxidative stress and that increased oxidative stress in accumulated fat is associated with dysregulation of adipocytokines and development of metabolic syndrome [6]. Oxidative stress has been shown to be involved in the process of atherogenesis [10], ischemic heart disease [11], obesity [6], metabolic syndrome or syndrome X, diabetes [12,13], as well as in immunodeficiency [14]. The stability of tissues against oxidative stress is however enhanced by antioxidant compounds, which could be present in the diet [15]. Antioxidants have also been shown to be very effective in inhibiting the oxidation of LDL and scavenging of free radicals and reactive oxygen species *in vitro* [16,17,18,19,20]. These compounds are present in fruits and vegetables, which contain natural antioxidants, and are known to delay the onset of atherogenesis.

Cissus quadrangularis (Linn) has been used by common folk in India for promoting the fracture healing process. It has been prescribed in Ayurveda as an alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, and in the treatment of irregular menstruation and asthma. In Cameroon, the whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant are important in the management of various ailments. Earlier works on *Cissus quadrangularis* report its effectiveness on the management of obesity and complications associated with metabolic syndrome [21], as well as its antioxidant and

free radical scavenging activity *in vitro* [22,23]. Various formulations now contain extracts of *Cissus quadrangularis* in combination with other compounds, used for the purpose of management of overweight and obesity, as well as complications resulting from these conditions, notably metabolic syndrome (syndrome X). Phytochemical analyses of *Cissus quadrangularis* revealed high contents of ascorbic acid, carotene, anabolic steroid substances, and calcium. The stem contains two asymmetric tetracyclic triterpenoids, and two steroid principles. The presence of β -sitosterol, δ -amyrin, δ -amyrone, and flavanoids (quercetin) has also been reported [24,25], all these components having potentially different metabolic and physiologic effects.

Although different uses of *Cissus quadrangularis* have been investigated, [26,27] the antioxidant potential of various *Cissus quadrangularis* formulations have not been evaluated in the modulation of obesity-induced oxidative stress. It was for this reason that the present study was designed, using a *Cissus quadrangularis* formulation (CORE) and CQR-300 (a standardized extract of *Cissus quadrangularis*, 2.5 % keto-steroids, and 15% soluble plant fiber).

Methods

The study was approved by the Cameroon National Ethics Board. The purpose, nature, and potential risks of the study were explained to all participants, who gave their written informed consent before participation. The study was conducted in accordance with the Helsinki Declaration (1983 version).

Sample

CORE (Table 1) was obtained from Soy Labs, LLC., Fairfield, California, USA;. CQR-300, a standardized extract of *Cissus quadrangularis* containing 2.5% keto-steroids and 15% soluble plant fiber (Gateway Health Alliances, Inc, Fairfield, California, USA).

***In vitro* Antioxidant potential of the CORE formulation and CQR-300**

Both CORE and CQR-300 were dissolved in acidified methanol prepared as previously described by Agbor et al. [19] for the *in vitro* antioxidant study. Three methods were used for the determination of antioxidant potential: Folin (polyphenol content), ferric reducing antioxidant power (FRAP, antioxidant power), 1,1-Diphenyl-2-picrilhydrazyl (DPPH, radical scavenging potential).

Polyphenol content – The Folin Ciocalteu reagent (Sigma Chemical Co., St Louis, MO, USA) was used to determine the concentration of polyphenol as a measure of antioxidant potential of CORE and CRQ-300. The reagent was diluted 10 times before used as described by Singleton et al. [28]. The absorbance was measured at 750 nm (Genesys Spectronic 20).

Ferric Reducing Antioxidant Power (**FRAP**) was measured as earlier described by Benzie and Strain. [29]. In brief, 2000 μ l of freshly prepared FRAP reagent (10 parts of 300 mM acetate buffer (pH 3.6), 1 part of 10 mM TPTZ (Sigma, in 400 mM HCl), and 1 part of 20 mM ferric chloride). After an initial incubation for 15 minutes at 37°C, the absorbance was read at 593 nm.

Scavenging potential against 1,1-Diphenyl-2-picrilhydrazyl (**DPPH**) measured the ability of the extracts to scavenge free radicals. 20 μ l of extract was introduced into 2 ml methanolic solution of DPPH (0.3 mM) and kept in the dark for 30 minutes. The extract was replaced by methanol for the control, and catechin was used as the standard. The absorbance was read at 517 nm, and the antioxidant content and percentage inhibition of the extract calculated as earlier described by Yen and Duh, [30].

In all three methods mentioned above, measurements were done in triplicates.

***In vivo* study**

Participants

A total of 168 overweight, obese, and normal weight participants aged between 19 and 50 years were selected from a group responding to a radio and poster advert. The BMIs of participants ranged from 25.0 to 48.7, and their weights ranged from

70.6 to 142 kg. After physical examination, which included measurement of blood pressure, participants with unusually elevated fasting blood glucose levels, those who were pregnant or lactating, as well as those on any form of weight-reducing medication were excluded from the study. Also excluded were participants who were involved in intense exercise programs, had medical conditions known to affect serum lipids, or had a history of drug or alcohol abuse. A total of 153 participants completed the study, while 15 participants dropped out of the study for personal reasons (7 moved out of town or had to travel during study period; 4 participants thought they had lost enough weight; while 4 participants were on malaria treatment and were excluded before the end of the study period).

Trial protocol

The study was double-blind placebo controlled, with the 168 overweight, obese, or normal weight participants of both sexes (between 19 and 50 years) distributed as outlined in Table 2. The participants who were either on their normal diet or on an energy restricted (2100 Kcal / day) diet, received two daily doses in the form of capsules of CORE for 8 weeks, and CQR-300 or placebo for 6 weeks. The capsules were identical in shape, color, and appearance, with neither the participants nor researchers knowing what capsule they received. Side effects were solicited on each weekly visit. Body weight and percentage body fat were determined in 12-hour fasted participants using a Tanita™ scale. Height was measured with a stadiometer to the nearest 0.5cm. Blood samples were obtained at the start and end of the trial period after a 12-hour overnight fast, into heparinized tubes, for the measurement of LDL oxidation (TBARS) [31] and protein carbonyl [32] content, total cholesterol, triacylglycerol, HDL-cholesterol, LDL-cholesterol, and glucose (cholesterol Infinity, triglyceride Infinity, EZ HDL™ cholesterol, EZ LDL™ cholesterol, Glucose Trinder) from SIGMA Diagnostics. Serotonin was measured using an enzyme immunoassay method (Serotonin EIA kit, BioSource Europe S.A, Belgium), creatinine by a modification of the method of Bartels et al. [33], while MDA was measured by a standard established method [34].

Statistical analyses

Statistical Package for the Social Sciences (SPSS) [35] software was used for all statistical analysis. The data were presented as means \pm SD. The statistical difference between samples was assessed by a Student's t-test for normal distribution or the Mann-Whitney test for non-normal distribution, after ANOVA testing of all the groups showed that significant differences existed. Paired Student's t-test was carried out on the start and end values of all the groups.

Results

In vitro antioxidant potential of CORE and CQR-300

The *in vitro* antioxidant capacity of CORE was significantly ($p<0.01$) higher than that of CQR-300 irrespective of the method of analysis used. Considering the composition of CORE (Table 1), it is likely that the other components present act synergistically with the *Cissus quadrangularis* extract present (Table 3).

Table 4 presents the effect of CORE and CQR-300 on the oxidative stress parameter (TBARS and carbonyls). CORE was more effective in reducing oxidative stress than CQR-300, the reduction being more obvious in the diet-restricted group. It significantly ($p < 0.01$) reduced the formation of TBARS and carbonyls compared to CQR-300.

Effect of CORE and CQR-300 on Body weight

Obese participants, who received CQR-300 (300mg daily), had significantly ($p<0.05$) greater reduction in body weight compared to those on placebo (Table 5). This reduction in body weight corresponded to a 5.4 % reduction in BMI. CORE had a more significant ($p<0.01$) effect (8.5% reduction for participants on an energy restricted diet) on the weight of participants compared to CQR-300. There was no significant net change in weight in participants on placebo during the study period.

Table 6 presents the effect of CORE and CQR-300 on blood lipids and fasting blood glucose levels. For participants on a restricted diet, six weeks use of CQR-300 reduced plasma total cholesterol by 18.0%, LDL-cholesterol by 29.0%, triacylglycerol by 21.7%, and fasting blood glucose by 14.6%. This treatment also increased the concentration of HDL-cholesterol by 21.1%. On the other hand, CORE (group 3) reduced the concentration of plasma total cholesterol by 26.0%, LDL-cholesterol by 32.4%, triacylglycerol 28.0%, and fasting blood sugar 16.1%. The CORE formulation also increased HDL cholesterol by 43.0%. The above mentioned changes were less obvious in participants whose diets were not restricted.

The effect of CORE and CQR-300 on malondialdehyde (MDA), serum serotonin, and creatinine levels are presented in Table 7. CQR-300 significantly ($p<0.05$) reduced the concentration of plasma MDA. This effect was accompanied by a slight increase in the urinary concentration of MDA though not significant. CQR-300 also significantly ($p<0.05$) increased the concentration of plasma 5-HT by 53.3% and plasma creatinine levels by 23.5%. An increase in 5-HT of 17.0% was also observed in the placebo group, while CORE (group 3) showed a significant increase of 39.1%. As such results for 5-HT and creatinine were significantly ($p<0.05$) lower for CORE than CQR-300.

Discussion:

The role of antioxidants from natural products in degenerative disease has attracted more interest on natural products research. In this study, we evaluated the antioxidant potential of CORE and CQR-300 on obesity-induced oxidative stress. The parent plant in these two formulations is *Cissus quadrangularis*. The formulation had a higher *in vitro* antioxidant potential than CQR-300 irrespective of the method used for the assay. The high antioxidant potential of CORE may be due to its composition. It contains some tea polyphenols and selenium that are potential antioxidants and thus complement the antioxidant potential of the parent plant in this formulation (*Cissus quadrangularis*). On the other hand, CQR-300 is a standardized extract of *Cissus quadrangularis* and no antioxidant was added to it.

Obesity may induce systemic oxidative stress, and increased oxidative stress in accumulated fat is one of the underlying causes of dysregulation of adipocytokines and development of metabolic syndrome [6]. Oxidative stress plays critical roles in the pathogenesis of various diseases [36]. In order to investigate if oxidative stress was increased in the obese participants, we measured lipid peroxidation (which represent the plasma TBARS) and the carbonyl compounds as markers of oxidative injury, which correlates with the BMI. The high plasma concentration of TBARS and carbonyl compounds was an indication of oxidative stress in the obese and overweight participants. These concentrations were significantly ($p<0.01$) reduced after treatment, with CORE being more effective than CQR-300. These samples may function through two mechanisms: either by scavenging free radicals to reduce oxidative stress, or by clearing the plasma of the products that are themselves potential oxidants. These activities may be attributed to the polyphenols present in the different formulations.

The use of CORE and CQR-300 during the study period brought about a significant reduction in the weight and BMI of obese patients. This loss in weight was comparable to that observed with cissus studies [21], sibutramine for one year [37], and orlistat for 6 months or 1 year [38,39]. This reduction in BMI was accompanied by an increase in HDL-cholesterol, and corroborates earlier work that showed an

inverse relation between BMI and HDL-cholesterol, the latter imparting possible health benefits in overweight and obese people [40,41]. The increase in the concentration of HDL-cholesterol and a decrease in the concentration of LDL-cholesterol could lead to a lowering of the atherogenicity and therefore a significant reduction in the potential incidence of coronary heart disease [42] (54% reduction of risk for a 0.6 mmol/L reduction of serum cholesterol) [43]. A reduction of fasting blood glucose levels as well as MDA levels have been previously reported to accompany weight loss in obese subjects [39]. The above observation could be linked to an increase in circulating creatinine and serotonin over the eight-week trial period. Serotonin is known to have a positive effect on mood and to reduce binge eating, which is common in obese people. Several previous studies [44,45] have shown a direct link between serotonin levels and weight loss. On the other hand, an increase in creatinine concentrations parallels an increase in lean muscle mass and a probable reduction in body fat.

Furthermore, *in vitro* studies (submitted in a different publication) show the ability of *Cissus quadrangularis* extracts to inhibit pancreatic lipase by approximately 60%, alpha-amylase by approximately 90%, as well as alpha-glucosidase by approximately 39%, all of which could contribute to weight reduction in obesity.

Conclusion

The CORE and CQR-300 (300 mg daily) brought about a significantly greater weight loss than placebo during the study period in obese individuals. This was accompanied by a significant improvement in the lipid profiles, blood sugar profiles, and serotonin profiles of study participants. They could have additional properties as antioxidants against oxidative stress in obese individuals. Thus, CQR-300 as well as CORE possesses antioxidant and free radical scavenging properties that could have applications in metabolic as well as other physiological complications in which there is an increase in oxidative stress.

These new findings warrant further exploration into the active phytonutrients of *Cissus quadrangularis* and the potential of its newly discovered weight loss and cardiovascular health benefits.

Authors' contributions

JO conceived, designed, and coordinated the work, as well as drafted the manuscript; DM and GF carried out analytical work; YS carried out analytical and statistical analyses of data; GA participated in the design and editing of the manuscript.

All authors have read and approved the manuscript.

Acknowledgments

The LNNB is grateful to Soy Labs (Fairfield, California, USA) for providing the cissus formulation (CORE) and AlbuMaSoy™, and to Gateway Health Alliances Inc. (Fairfield, California, USA) for preparing and supplying the *Cissus quadrangularis* extract (CQR-300).

References

1. McGill H.C, Jr, C. McMahan A, Herderick E.E, Zieske A.W, Malcom G.T, Tracy R.E, Strong J.P. Obesity Accelerates the Progression of Coronary Atherosclerosis in Young Men. *Circulation* 2002;105. <http://www.circulationaha.org>.
2. Montague, C.T., and O'Rahilly, S. The perils of portliness: causes and consequences of visceral adiposity. *Diabetes*. 2000, 49:883-888.
3. Matsuzawa, Y., Funahashi, T., and Nakamura, T.. Molecular mechanism of metabolic syndrome X: Contribution of adipocytokines adipocyte-derived bioactive substances. *Ann. N. Y. Acad.* 1999, *Sci.* 892:146-154.
4. Spiegelman, B.M., and Flier, J.S.. Obesity and the regulation of energy balance. *Cell*. 2001, 104:531-543.
5. Kahn, B.B, and Flier, J.S.. Obesity and insulin resistance. *J. Clin. Invest.* 2000, 106:473-481.
6. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, and Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J. Clin. Invest.* 2004, 114:1752-1761
7. Ford, E.S., Giles, W.H., and Dietz, W.H. Prevalence of the metabolic syndrome among US adults: findings from the Third National Health and Nutrition Examination Survey. *JAMA*. 2002, 287:356-359.
8. Isomaa, B., Almgren, P., Tuomi, T., Forsen, B., Lahti, K., Nissen, M., Taskinen, M., Groop, L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001, 24:683-689.
9. Grundy, S.M., Brewer, H.B., Cleeman, J.L., Smith, S., Lefant, C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation*. 2004;109:433-438.
10. Brown Rk, Kelly FG. Evidence for increased oxidative damage in patients with cystic fibrosis. *Paediatr Res* 1994; 36:487-93.

11. Gutteridge JM, Swain J. Lipoprotein oxidation: the 'fruit and vegetable gradient' and heart disease. *Br J Biomed Sci.* 1993, 50:284-8.
12. Mustafa A., David E., and Laa Ksonen. Diabetes, oxidative stress and physical exercise. *Journal sports Sc. Med.* 2002; 1: 1-14
13. Lean M. and Burns J. Tentatives pharmacologiques et nutritionnelles pour corriger le stress oxydant. Flammarion, medecine science journée de diabetologie 2001; 87-96
14. Ngondi JL, Oben J, Musoro FD, Etame SLH, and Mbanya D. The effect of different combination therapies on oxidative stress markers in HIV infected patients in Cameroon. *Aids Research and Therapy*, 2006, 3:19.
15. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL.. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N. Eng. J. Med.* 1989, 320: 915-924.
16. Frankel E.N., Waterhouse A.L., and Teissedre P.L. Principal Phenolic Phytochemicals in Selected California Wines and Their Antioxidant Activity in Inhibiting Oxidation of Human Low-Density Lipoproteins, *J. Agric. Food Chem.* 1995, 43, 890-894.
17. Kanner J.b, Frankel J.E, Granit R.B.G, and Kinsellatss J.E. Natural Antioxidants in Grapes and Wines, *J. Agric. Food Chem.* 1994, 42, 64-69.
18. Teissedre P. L., Frankel, E. N., Waterhouse, A. L., Peleg, H. & German, J. B. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* 199670: 55–61.
19. Agbor A. Gabriel, Julius E Oben, Jeanne Y Ngogang, Cai Xinxing, Joe A Vinson.: Antioxidant Capacity of Some Herbs/spices from Cameroon. A Comparative study of two Methods. *J. Agri and Food Chem.* 2005, 53: 6819-6824.
20. Agbor A. Gabriel, Joe A Vinson, Julius E. Oben, Jeanne Y Ngogang, comparative study of the in vitro antioxidant activity of white and black pepper, *Nutr. Res* (in press)

21. Oben J, Kuate D, Agbor G, Momo C, and Talla X. The use of a *cissus quadrangularis* formulation in the management of weight loss and metabolic syndrome. *Lipids in Health and Disease*, 2006; 5: 24
22. Agbor A. Gabriel, Kuate Dieudonne, Oben E. Julius E, Medicinal plants can be good source of antioxidant: Case study of Cameroon, *Pak. J. Biol. Sci.* 2006,
23. Mallika J and Shyamala CSD, *In vitro* and *In vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*. *Afri. J. of Biomed. Res*, 2005, 8, 95-99.
24. Mehta, M, Kaur, N., Bhutani, K. Determination of marker constituents from *Cissus quadrangularis Linn.* and their quantitation by HPTLC and HPLC. *Phytochem Anal*; 2001, 12: 91-105.
25. Jakikasem, S., Limsiriwong, P., Kajsongkarm, T., Sontorntanasart, T. Phytochemical study of *cissus quadrangularis*. *Thai J. Pharm.Sci.*, 2000, 24:25.
26. Chidambara Murthy KN, Vanitha A, Mahadeva Swamy M, Ravishankar, GA. Antioxidant and antimicrobial activity of *cissus quadrangularis* L. *Journal of Medicinal Foods* 2003; 6(2): 99-105.
27. Chopra S.S, Patel MR, Gupta LP, Datta IC. Studies on *Cissus quadrangularis* in experimental fracture Repair: Effect on Chemical Parameters in Blood. *Ind. J. Med. Res*, 1975, 63:6
28. Singleton VL, Orthofer R, Lamuela-Raventòs RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 1999, 299, 152-178.
29. Benzie IFF, Strain, J.J.. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power : The FRAP assay. 1996, *Anal.Biochem.* 239, 70-76.
30. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. *J. Agri. and Food Chem.* 1994, 42: 629–632.
31. Okhawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Annals Biochemistry*, 1979, 95:351-358.

32. Levine RL, Garland D, Oliver CN. The determination of carbonyl content in oxidatively modified proteins. *Methods in Enzymology*, 1990, 186: 484-477
33. Bartels, H., M. Boemer, and C. Heirli. Serum creatinine determination without deproteinization. *Clin. Chim. Acta* 1972 37:193–197
34. Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. *Biochemical Medicine*, 1976; 15: 212-216.
35. SPSS for Windows, Rel. 11.0.1. 2001. Chicago: SPSS Inc.
36. Brownlee M. 2001. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 414:813-820
37. Smith IG, Goulder MA. Randomized placebo-controlled trial of long-term treatment with sibutramine in mild to moderate obesity. *J Fam Pract*, 2001; 50: 505-512.
38. Kelly DE, Bray GA, Pi-Sunyer FX, Klein S, Hill J, Miles J, Hollander P. Clinical efficacy of orlistat therapy in overweight and obese patients with insulin-treated Type 2 diabetes. *Diabetes Care*, 2002; 25 (6): 1033-1041.
39. Yesilbursa D, Serdar Z, Serdar A, Sarac M, Jale C. Lipid peroxides in obese patients and effects of weight loss with orlistat on lipid peroxides levels. *International Journal of Obesity*; 2005; 29: 142-145.
40. Pietrobelli A, Lee RC, Capristo E, Deckelbaum RJ, Heymsfield SB. An independent, inverse association of high-density-lipoprotein-cholesterol concentration with nonadipose body mass. *Am J Clin Nutr* 1999; 69:614-620.
41. Knuiman JT, West CE, Burema J. Serum total and high density lipoprotein cholesterol concentrations and body mass index in adult men in 13 countries. *Am J Epidemiol* 1982; 116: 631-642.
42. Griffin BA. Lipoprotein and atherogenicity: An overview of current mechanisms. *Proceedings of the Nutrition Society* 1999; 58: 163-169.
43. World Health Organisation Guidelines-International Society of Hypertension Mild Hypertension Liaison Committee. 1999 World Health Organisation –International Society of Hypertension guidelines for the management of hypertension. *J. Hypertens* 1999; 17: 151-183.

44. Nelson A., Day, R., Glickman-Weiss, E., Hegstad, M., Sampson, B. Creatine supplementation raises anaerobic threshold. *FASEB Journal*, 1997;11, A589. (Abstract)
45. Rockwell JA, Rankin JW, Toderico B. Creatine supplementation affects muscle creatine during energy restriction. *Med Sci Sports Exer*. 2001 ;33(1):61-8.

Table 1. Detailed composition of the cissus formulation (CORE).

Item	Description	Active Amount Per Capsule (mg)	Amount of Active Per Day (2 capsules) (mg)
<i>Cissus quadrangularis</i>	5% ketosteroids	7.5	15
ChromeMate*	Niacin-Bound Chromium (10% Cr) -- Concentrate	0.15	0.3
Green Tea Extract (High Caffeine)	40% Polyphenols, 22% EGCG, 40% Caffeine	100	200
Selenium	0.5% L-Selenomethionine	0.06	0.12
AlbumaSoy**	Soy Albumin	50	100
Vitamin B6	pyridoxine hydrochloride	50	100
Vitamin B12	cyanocobalamin	0.05	0.1
Folic Acid	Folic acid	0.4	0.8
Total Per Unit		104.08	265.645

*ChromeMate is a trademark of InterHealth N.I.

**AlbumaSoy is trademark of Soy Labs, LLC

Table 2. Distribution of participants into treatment groups. The number given in parentheses represents the participants whose complete measurements were done over the study period.

Group No.	Participants	Treatment	No. of Participants
1	Males /Females BMI>30	Placebo 6 weeks (2100 Kcal/day diet)	33 (32)
2	Males /Females BMI>30	CORE 8 weeks (no dietary restriction)	33 (30)
3	Males /Females BMI>30	CORE 8 weeks (2100 Kcal/day diet)	35 (31)
4	Males /Females BMI>30	CQR-300 6 weeks (2100 Kcal/day diet)	35 (32)
5	Males /Females BMI 25 -29.9	CORE 8 weeks (no dietary restriction)	32 (28)

Table 3. *In vitro* antioxidant capacity of the CORE and CQR-300 (mg of catechin equivalent/ gram dry weight) by FRAP, Folin, and DPPH methods.

	FRAP Antioxidant (mg/g)	Folin Total Polyphenol (mg/g)	DPPH Antioxidant (mg/g)
CORE	22.67 \pm 4.58	56.70 \pm 6.12	8.46 \pm 1.30
CQR-300	15.85 \pm 3.08*	42.33 \pm 3.21*	5.97 \pm 0.66*

* $p < 0.01$ compared to the *cissus* formulation for each method.

Table 4. The effect of the CORE and CQR-300 extracts on the concentrations of TBARS and carbonyls.

	Group 1 BMI>30 Placebo (2100 Kcal/day diet)		Group 2 BMI>30 CORE (no dietary restriction)		Group 3 BMI>30 CORE (2100 Kcal/day diet)		Group 4 BMI>30 CQR-300 (2100 Kcal/day diet)		Group 5 BMI 25-29.9 CORE (no dietary restriction)	
Time (weeks)	T=0	T=6	T=0	T=8	T=0	T=8	T=0	T=6	T=0	T=8
TBARS (umol/L)	1.42 \pm 0.51	1.60 \pm 0.38	1.66 \pm 0.56	0.87 \pm 0.53**	1.78 \pm 0.38	0.72 \pm 0.35** [†]	1.84 \pm 0.51	0.98 \pm 0.18**	1.06 \pm 0.24	0.66 \pm 0.13** [†]
Carbonyls (nmol/mg protein)	1.95 \pm 0.73	2.09 \pm 0.68	2.03 \pm 0.39	0.98 \pm 0.39**	1.96 \pm 0.40	0.84 \pm 0.27** [†]	2.06 \pm 0.28	1.10 \pm 0.34**	0.94 \pm 0.20	0.77 \pm 0.36** [†]

** $p < 0.001$ compared to T=0; [†] $p < 0.01$ compared to CQR-300 (Group 4).

Table 5. Effect of the CORE and CQR-300 on body weight, BMI, and Body fat

	Group 1 BMI>30 Placebo (2100 Kcal/day diet)		Group 2 BMI>30 CORE (no dietary restriction)		Group 3 BMI>30 CORE (2100 Kcal/day diet)		Group 4 BMI>30 CQR-300 (2100 Kcal/day diet)		Group 5 BMI 24-29.9 CORE (no dietary restriction)	
Time (weeks)	T=0	T=6	T=0	T=8	T=0	T=8	T=0	T=6	T=0	T=8
Weight (kg)	112.4 ± 2.6 (1.1)	113.6 ± 2.0	95.8 ±11.8	89.2 ±9.2 (6.9)*	95.3 ±14.6	87.2 ±8.9 (8.5)**†	118.6 ±3.8	113.8 ±2.5 (4.0)*	76.3 ±6.8	72.5 ±4.7 (5.0)*
BMI (kg/m ²)	38.1 ±1.1	38.0 ±0.9	37.7 ±6.3	34.6 ±8.6	37.5 ±4.7	33.8 ±6.9	38.8 ±1.0	36.7 ±3.4 .5	27.3±2 3.0	26.3±
Body fat (%)	43.6 ±1.6	42.8 ±2.1	46.5 ±3.1	43.7 ±2.4	48.1 ±4.4	44.3 ±2.1	44.3± 5.4	42.1 ±3.6 ±2.2	35.9 34.2	34.2 ±3.0

Values are means ± SE.

p*<0.05, *p*<0.01, compared to placebo, †*p*<0.01 compared to CQR-300 (Group 4),

() = percentage change in weight.

Table 6. Effect of the CORE and CQR-300 on blood lipids and fasting blood glucose

	Group 1 BMI>30 Placebo (2100 Kcal/day diet)		Group 2 BMI>30 CORE (no dietary restriction)		Group 3 BMI>30 CORE (2100 Kcal/day diet)		Group 4 BMI>30 CQR-300 (2100 Kcal/day diet)		Group 5 BMI 24-29.9 CORE (no dietary restriction)	
Time (weeks)	T=0	T=6	T=0	T=8	T=0	T=8	T=0	T=6	T=0	T=8
Total Cholesterol (mg/dL)	136.3 ±9.0	138.3 ±11.1	159.1 ±14.6	116.2 ±9.7*	171.0 ±15.9	126.5 ±7.9 *†	138.3 ±13.8	113.4 ±4.5*	152.6 ±8.8	123.0 ±4.7*
LDL-cholesterol (mg/dL)	88.4 ±5.9	88.2 ±5.9	99.8 ±6.5	81.4 ±1.7	116.6 ±5.4	78.8 ±3.3	92.7 ±7.0	65.8 ±3.4	101.6 ±1.9	74.8 ±0.9
HDL-cholesterol (mg/dL)	27.3 ±2.2	25.8 ±3.1	36.6 ±4.7	55.1 ±6.4*	38.6 ±10.5	55.2 ±8.6*†	25.6 ±4.8	31.0 ±3.9	44.4 ±7.7	52.0 ±8.1
Triacylglycerol (mg/dL)	93.6 ± 4.8	90.5 ±10.4	156.0 ±16.8	95.6 ±8.1	144.9 ±43.3	104.3 ±26.2*†	93.8± 9.7	73.4 ±6.0***	117.4 ±9.8	99.8 ±11.2
Fasting Blood glucose (mg/dL)	93.6 ±7.2	89.4 ±10.1	101.3 ±8.6	87.7 ±10.0	102.4 ±1.8	85.9 ±5.2	91.8 ±6.9	78.4 ±11.2	93.3 ±10.2	82.7 ±7.2

Values are means ± SE, comparing starting point to end point, significant differences were at * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ for the same treatment.

Table 7. Effect of the CORE and CQR-300 on plasma and urinary malondialdehyde, 5-HT, and plasma creatinine levels

	Group 1 BMI>30 Placebo (2100 Kcal/day diet)		Group 2 BMI>30 CORE (no dietary restriction)		Group 3 BMI>30 CORE (2100 Kcal/day diet)		Group 4 BMI>30 CQR-300 (2100 Kcal/day diet)		Group 5 BMI 24-29.9 CORE (no dietary restriction)	
Time (weeks)	T=0	T=6	T=0	T=8	T=0	T=8	T=0	T=6	T=0	T=8
Plasma MDA (μ mol/L)	1.8 \pm 0.1	1.7 \pm 0.1	1.8 \pm 0.6	1.0 \pm 0.5*	1.9 \pm 0.9	1.1 \pm 0.6 *†	1.6 \pm 0.2	1.0 \pm 0.4*	1.8 \pm 0.3	1.2 \pm 0.5*
Urinary MDA (μ mol/day)	3476.2 \pm 211.7	3492.2 \pm 188.4	3864.3 \pm 238.4	4325.1 \pm 167.5*	4022.1 \pm 148.1	4485.8 \pm 203.3	3416.2 \pm 110.9	3581.5 \pm 200.2	3593.8 \pm 116.5	3971.0 \pm 104.5
Plasma 5- HT (mg/l/dl)	30.6 \pm 1.9	35.8 \pm 2.1	32.5 \pm 1.9	42.4 \pm 3.4*	38.4 \pm 1.5	53.4 \pm 3.6†	35.4 \pm 1.7	54.3 \pm 2.9*	33.1 \pm 3.7	43.2 \pm 2.1*
Plasma creatinine (mg/dl)	25.7 \pm 3.8	27.3 \pm 1.4	25.9 \pm 1.8	31.8 \pm 2.1	24.4 \pm 3.2	28.9 \pm 1.2	27.2 \pm 2.2	33.6 \pm 1.4*	29.0 \pm 0.8	33.8 \pm 1.2

Values are means \pm SD, comparing starting point to end point, significant differences were at * p <0.05 for the same treatment.