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Modulatory effect of a polyphenolic rich extract of Dacryodes macrophylla berries on biomarkers of metabolic syndrome and oxidative stress in rats fed High Fat- High Sucrose diet

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ABSTRACT

Bioactive compounds in fruits have been associated with improvement of markers of metabolic syndrome as well as the prevention of oxidative damage. The aim of this study was to evaluate the preventive effect of the extract of *Dacryodes macrophylla* against weight gain and oxidative stress in *Wistar* rats fed with a High Fat-High Sucrose (HFHS) diet. Changes in body weight, lipid profile, transaminases, creatinine, oxidative stress markers, catalase, nitric oxide, and total antioxidant capacity of plasma (TAC) were evaluated. To achieve this, the hydroethanolic of *D. macrophylla* fruits was prepared, by maceration in the water: ethanol(1:1v/v), for 48hrs. The obtained extract was used to evaluate its preventive effect against weight gain/obesity and oxidative stress in rats fed with HFHS diet for 45days. The HFHS diet was observed to increase body weight, plasma triglyceride (TG), Total Cholesterol (TC), Malondialdehyde (MDA), nitric oxide (NO) levels and decrease thiol proteins and catalase activity. Treatment, especially with the 200mg/kg/bw dose of hydroethanolic extract of *Dacryodes macrophylla* (DMHE) reversed the effects of the diet on these parameters. From these results it can be concluded that the crude organic extract of *Dacryodes macrophylla* berries could have anti-obesity/overweight and antioxidant properties.

Keywords: Weight gain, Oxidative stress, *Dacryodes macrophylla*, High Fat-High Sucrose diet.

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INTRODUCTION

Obesity has been defined as a condition in which adipose tissue increases and can be defined as an increase in body weight that results from excessive fat accumulation[1]. On a global scale, it has reached epidemic proportions and is a major contributor to the global burden of chronic diseases and disability. Currently, more than one billion adults worldwide are overweight and at least 300 million of them are clinically obese[2]. The incidence of obesity has risen alarmingly over the last few decades[3] and it was estimated in 2005 that its prevalence after age adjustment ranges from 13.1% to 30.0% [4]. The excess accumulation of body fat referred to as overweight or obesity, depending on its extent, results mainly from a chronic disequilibrium between food consumption and energy expenditure[5]. Individuals with obesity are at increased risk of diabetes, cardiovascular diseases and cancers, and usually die prematurely[6].

Overweight people often exhibit perturbed energy and lipid metabolism characterized by elevated blood glucose, triglyceride and low density lipoprotein (LDL) levels[7]. Obesity is also associated with an increased oxidative damage on cellular constituents (proteins, lipids and DNA) and increased inflammation as indicated by elevated levels of tumor necrosis factor (TNF), interleukin-1β and other pro-inflammatory cytokines which predispose for several major age-related diseases including diabetes[8], cardiovascular diseases, stroke[9] and possibly cognitive impairment and Alzheimer's disease. Lifestyle changes, such as regular physical activity and nutrition improvement, are the bases for successful long-term weight loss and control of overweight and obesity. Among dietary changes, indirect evidence from a number of epidemiological studies suggests a beneficial role of foods such as beans, vegetables and fruits such as berries.

Numerous epidemiological studies have provided results that emphasize the importance of fruits and vegetables as being part of a daily diet for better health[10]. Most of the phytonutrients found in fruits and vegetables are antioxidants. A large clinical study provided results in support of recommendations to consume antioxidant-rich foods to reduce the risk of metabolic syndrome[11]. Berries are emerging as a dietary source of multiple phytonutrients[12], including polyphenolic compounds. Studies using chokeberries, cranberries, blueberries, and strawberries , or purified anthocyanin extracts have demonstrated significant improvements in LDL oxidation, lipid peroxidation, total plasma antioxidant capacity, dyslipidemia, endothelial function and glucose metabolism as well as their ability to induce satiety/counteract overweight and inhibit adipogenesis [12,13].

A number of different naturally occurring antioxidants have been identified from vegetables, fruits and spices which act powerfully. Cameroon has a lot of foodstuff such as fruits, vegetables and spices which could be potentially used as sources of antioxidants, and hence serve as therapeutic agents in the management of obesity and related complications. The aim of this study was to investigate the potential use of polyphenolic rich extracts of a Cameroonian wild berriy (CWB): *Dacryodes macrophylla* in the prevention of weight gain and oxidative stress.

MATERIAL AND METHOD

Plant material

The berries of *Dacryodes macrophylla* were harvested in Ngoumou in the Center Region of Cameroon in the month of August 2012. It was identified at the National Herbarium in Yaounde, Centre Region, Cameroon. The fruits were selected on the basis of color intensity, washed and a sharp knife was used to open the fruits in order to facilitate drying. The fruits were dried in an oven at 60°C for about 14 days. The dried fruits were put in a plastic bag for conservation.

Extract preparation

The skin (integument) and pulp of the dried fruit were separated from the seed, ground and extracted in our laboratory using water: ethanol (50:50 v/v) as solvent. After 48 hours of maceration, the extracts were filtered using Whatman #2 filter paper (Whatman International Limited, Kent, England) using a funnel and concentrated to about 10% of the original volume by a rotavapor before drying in an oven at 50°C.

Animal experimentation procedure

The Composition of 100g of experimental diet was as follow:

Normal Caloric Diet: Protein: 20%; corn oil: 10%; Starch: 50%; Saccharose: 3%; fibers: 2%; Vitamins and Minerals: 5%; Distilled water : 10%.

High Fat-High sucrose Diet: Protein: 15.9%; Animal fat: 26.9%; Starch: 25.6%; Saccharose: 18.6%; Fibers: 2%; Vitamins and Minerals: 3.2%; Distilled water: 10%.

Treatment Schedule:

For this study, 25 male albino *Wistar* rats weighing between 200-280 g were obtained from the animal house of the Laboratory of Biochemistry, Department of Biochemistry, University of Yaounde I, Cameroon. The study protocol was approved by the animal ethical committee of University of yaounde I.

Animals were acclimated for a period of 15 days before treatment. They were weighed and divided into 5 groups of 5 rats.

Group 0 (NCD):

Normal caloric diet + distilled water

Group I (HFHSD):

High Fat-High Sucrose diet + distilled water daily

Group II (HFHSD DM 200mg):

High Fat-High Sucrose diet + 200mg/kg bw of HE extract of *D. macrophylla*

Group III (HFHSD DM 400mg):

High Fat-High Sucrose diet + 400mg/kg bw of HE extract of *D. macrophylla*

Group IV (HFHSD STA 10mg):

High Fat-High Sucrose diet + 10mg/kg bw of Statine

Extracts or distilled water (depending on the group) was administered by oral gavage every day. The body weight and fasting blood glucose levels of all rats were recorded at regular intervals during the experimental period. At the end of the 45 days experimental period, they were sacrificed and the blood collected in EDTA tubes for plasma and hemolysate preparation. The rats were further dissected to collect vital organs notably; the heart, liver and kidney which were weighed and a 10 % homogenates of each organ prepared. All these were preserved at -20°C and later used for biochemical assays. The schedules and procedures were performed in the experimental animal house of the Laboratory of Biochemistry of the University of Yaounde I, Cameroon. The study protocol was approved by institutional animal ethical committee.

Preparation of biological samples

The whole blood of rats in EDTA tubes from the jugular artery a. This blood was allowed to stand for about 2hrs after which it was centrifuged at 3400 rpm for 10 minutes. The supernatant constituting the plasma was collected into dry Eppendorf tubes and stored at -20°C [14].

Preparation of hemolysate of erythrocytes

Whole blood (100µl) was washed in 2ml 0.9% NaCl and centrifuged at 3400rpm for 10 minutes. This process was repeated twice. Hemolysis was later achieved by adding 2ml of distilled water to the washed erythrocytes and the hemolysate conserved in Eppendorf tubes and stored at -20°C for determination catalase activity [14].

Preparation of tissue homogenates

Homogenates were prepared at a concentration of 10% by grinding a given amount of tissue in the corresponding amount of 0.9% (w/v) NaCl. The resulting homogenate was centrifuged at

3400rpm for 10minutes and the supernatant was conserved in Eppendorf tubes and stored at -20°C for later use in biochemical assays[14].

BIOCHEMICAL ANALYSIS

Plasma glucose was estimated by the GOD-PAP method[15], triglycerides by the GOP-PAP method[16], total cholesterol by the CHOD-PAP method described by Richmond[17], HDL cholesterol by the CHOD-PAP method[18]. Plasma and tissue (heart, liver, and kidney) MDA concentrations were evaluated as described by Gutteridge and Wilkins[19]. Plasma, erythrocyte and tissue (heart, liver, kidney) thiol groups were assayed as described by Ellman[20]. Catalase activity was determined as described by Sinha[21]and nitric oxide (NO) by the method of Sreejayan and Rao[22]. Plasma alanine aminotransferase (ALAT), Aspartate aminotransferase activities and creatinine level were determined by the kinetic UV method[23].

Statistical analysis

Statistical analyses were done using the Statistical Package for Social Sciences (SPSS) software, version 10.0 for Windows. Results were expressed as mean ± SEM. Analysis of variance (ANOVA) between groups was done at one factor. A Post hoc test of Least Significant Difference (LSD) was used to compare means. *Indicates a significant difference between the positive and the negative control groups, meanwhile a, b and c indicate significant differences between positive control groups and the treated groups. Results were considered significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

The prevalence of metabolic disorders, such as obesity, hyperlipidemia and hyperglycemia, is rising dramatically in developing nations. Obesity is reaching epidemic proportions worldwide[24] and is an established risk factor for various co-morbidities, such as type 2 diabetes mellitus and cardiovascular diseases[25]. The development of obesity induces systemic oxidative stress[26] and affects inflammatory state[27]. Developing preventive and therapeutic solutions that impede the rise in metabolic disorders has become a primary goal in the past decade. In addition to pharmaceutical approaches, the use of natural products at physiological doses has been recognized as an effective regimen to improve several health conditions[28]. Plant-based treatments have been validated as strategies in the prevention of obesity and type 2 diabetes mellitus. Thus this study was designed to investigate the potentials of a Cameroonian berry to improve clinical conditions of metabolic syndrome

Table 1 below is a representation of the preventive effect of the hydroethanolic extracts of DM berries against weight gain and abdominal fat accumulation.

The body weight between the HFHS diet and normal diet groups began to differ significantly after 14 days of treatment. Weight gain was lower in groups fed with HFHS diet and supplemented with DM extracts, though not significant. This effect was associated with a high abdominal fat accumulation in the positive control group. A significant decrease was observed in the groups supplemented with statine compared to the positive control ($p<0.05$). The significant difference observed between the treated (HFHS + statine/DMHE) and untreated HFHS ($p<0.05$) groups indicated that the HFHS diet significantly increased the body weight of rats as well as the organ to body weight ratio of the liver and kidney over a period of 6 weeks. The significant increase of liver weight resulted from the accumulation of lipids by lipogenesis. Sucrose consumption increases caloric intake through an up regulation by the hypothalamus[29]. Our results are consistent with previous studies[30] which proposed that the consumption of high-fat diets is markedly associated with dyslipidemia and consequently a reduction of insulin sensitivity.

As concerns the preventive effect of DM extract against elevation of lipid profile markers and fasting blood glucose, a significant difference between NCD and HFHSD groups in the values of triglycerides and total cholesterol was observed ($p<0.05$). This indicated that the high fat-high sucrose diet significantly increased triglycerides and total cholesterol in animals through out the experiment. After administration of extracts and the reference drug (Statine), it was observed that DMHE 400mg/kg bw showed a better significant reducing effect compared to DMHE 200mg/kg bw and Statine ($p<0.05$). This study also revealed an increase in blood glucose though not significant in the HFHS group as compared to the NCD group. Supplementation of the HFHS diet with DMHE showed no significant reducing effect on blood glucose after six weeks.

Rats fed with a high fat-high sucrose diet showed a significant increase in blood TG and TC levels, as previously reported[31]. This is explained by the fact that when sucrose is consumed, it is broken down to glucose and fructose. Absorbed fructose is converted to lipogenic precursors leading to an increase in plasma triglyceride levels[32]. High plasma TC is suggested as an important factor for the development of inflammation in mice[33]. In the present study, the high fat diet elevated TG and TC levels significantly ($p<0.05$) in the HFHS group. These findings are in accordance with the results of Yang *et al.* [34], who reported significant increases in serum TG and TC in rats fed a high-fat diet. Therefore hydroethanolic extracts of *D. macrophylla* had strong

hypotriglyceridemic and hypocholesterolemic effects on the plasma of rats. These results are contrary to those obtained by Erlund *et al.*[35] who found that, berry supplementation did not significantly affect total cholesterol and triglycerides, but HDL concentrations significantly increased compared to the control.

It was observed that the blood glucose level in rats fed on high sucrose diets was not significantly affected. Neither plasma glucose nor insulin levels were significantly higher. This corroborated with the finding of Fukuchi *et al.* [36] and Torronen *et al.*[37], who showed that berry supplementation did not significantly affect glucose and insulin concentrations.

Previous studies have shown that feeding rats with a high fat high fructose diet, increased proinflammatory and oxidative stress markers, and decreased antioxidant defenses[38,39].

One of the objectives of this study was to evaluate the preventive effect of the tested extract against oxidative damage induced by the experimental diet. .

Table 3 below depicts the effect of *D. macrophylla* extracts on the concentration of malondialdehyde in different tissues in rats after 45 days experimentation. A significant increase ($p<0.05$) in the levels of malondialdehyde was observed in both NCD and HFHS groups. This showed that the diet administered to the rats established oxidative stress in the plasma, heart and especially the liver and kidneys as reported by Noeman *et al.*[40]

Rats fed with HFHS diet associated with DMHE for 6 weeks, showed lower MDA levels ($p<0.05$) in plasma, liver heart and kidney compared to the HFHS group. In addition, liver, heart and kidney MDA levels in the HFHS + DMHE 200mg/kg bw group were lower than in the HFHS + DMHE 400mg/kg bw group but the effect was similar in liver. This result is similar to previous findings on berries that showed that strawberry anthocyanins increased antioxidant capacity and decreased lipid oxidation[41]. This implies that extracts are endowed with an antioxidant potential to block the transformation of the primary products (hydroperoxides) of oxidative stress into the secondary products (MDA) which are very harmful to cell membranes. This is due to the presence of polyphenols, particularly flavonoids, which significantly reduces MDA during oxidative stress as reported by Coimbra *et al.*[42] in the study of green tea for its chemoprotective action in screening against the deleterious action of free radicals.

The level of thiol groups in rats fed HFHS diet and the same diets supplemented with medicines (Statine, DMHE) are shown in the Table 4 below. Thiol protein groups were significantly lower ($p< 0.05$) in all tissues in the HFHS group than in the NCD group. In the liver, plasma, kidney, thiols levels were observed to be higher in the HFHS + DMHE after six weeks of extract

administration. In addition the HFHS + DMHE 400mg/kg bw group showed better results compared to the HFHS + DMHE 200mg/kg bw.

The effect of DM on erythrocyte catalase level and plasma and heart nitric oxide level are represented in Table 5 below. Catalase activity was significantly lower ($p< 0.05$) in the HFHS group than in the NCD group Catalase results were found to be similar to thiol protein results as supplemented groups were found to have higher catalase activities after an experimentation period of six weeks. When obesity persists for a long time, antioxidant sources can be depleted, decreasing the activity of enzymes such as superoxide dismutase (SOD) and catalase (CAT)[43]**.** For the enzymatic antioxidant system, a non significant increase in catalase activity was observed in erythrocytes after the administration of DMHE extract. This suggests that the composition of berries contain compounds that increase the antioxidant capacity or compounds that act to increase production of endogenous antioxidant sources[44]. Thus, supplementation with antioxidants would reduce the risk of complications related to obesity and oxidative stress[45]. The increase in thiol protein concentration of the treated group compared to the HFHS group at the level of the plasma indicated that the diet had an effect in reducing the antioxidant capacity in plasma through the establishment of oxidative stress as observed by Vincent *et al.*[46] who reported that the level of serum antioxidants such as glutathion is decreased in obesity. The increase in thiol protein at the level of the plasma compartment in treated groups, particularly in the DMHE 400mg/kg bw group showed that the extract has an antioxidant capacity in accordance with Molan *et al.*[47] who demonstrated a significant increase in the serum antioxidant potential of Sprague Dawley rats following six days of blueberry consumption, indicating the ability to elevate circulating antioxidant potentials *in vivo*.

The supplementation of the HFHS with DMHE or reference drug led to a significant decrease in the level of NO in the both body compartments. In addition, DMHE 200mg/kg bw showed a greater effect on NO level than DMHE 400mg/kg bw. The role of Nitric oxide (NO) as physiological regulator of many functions in cardiovascular, neuromuscular, neurological, genitourinary, gastrointestinal, and renal tissues has been highlited in many reports. Inhibitors of nitric oxide synthase reduce NO production and prevent the decrease in insulin secretion caused by free fatty acids [48].

The concentration of creatinine and the activity of transaminases (ASAT and ALAT), reflect the degree of renal and hepatic defect in the course of damages generated by exposure to certain cardiovascular disease risk factors[49]. These can lead to diverse complications. These markers

have been used as indicators of renal and hepatic dysfunctions. Table 6 below shows the effect of berries on hepatic and renal toxicity markers.

Table 1: Effect of subacute administration hydroethanolic extract of *D. macrophylla* berries on weight parameters after 6 weeks.

	Body Weight (kg)				Abdominal fat (% body weight)
Groups	**Day 0**	**Day 14**	**Day 28**	**Day 45**	
NCD	239.06±5.34	240.68±4.28	243.18±3.88	245.16±5.98	
HFHSD	250.33±19.39	259.33±16.56 (3.69%)	265±19.69 (5.88%)	295.33±25.32 (17.94%)	3.55±0.72
HFHS. DM (200mg/kg bw/day)	252,50±9,57	258,50±21,99 (2,25%)	257,75±22,89 (1,94 %)	264±25,30 (4,42 %)	3,88±1,00
HFHS. DM (400mg/kg bw/day)	248,00±12,16	252,00±8,71 (1,89%)	250,00±1,00 (0,97%)	262±2,00 (5,79%)	2,24±0,58
HFHSD. Statine (10mg/kg bw/day)	254,40±18,83	251,00±31,96 (-1,48%*)	244,20±37,08 (-4,08%)	239±32,57 (-5,82%)	1,66±0,45*

*Indicates significant difference between positive and negative control groups

Table 2: Effect of extracts on the lipid profile of rats fed High Fat- High Sucrose diet

Group	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	Blood glucose (mg/dL)
NCD	54.416±2.50*	67.75±5.05*	52.77±12.65
HFHSD	164,75±15.32[b]	90.98±13.37[b]	74.80±15.18[a]
HFHS. DM (200mg/kg bw/day)	72.45±17.48[a]	64.91±12.01[a]	65±8.98[a]
HFHS. DM (400mg/kg bw/day)	51.34±4.79[a]	59.16±16.49[a]	74.33±5.85[a]
HFHSD.Statine (10mg/kg bw/day)	78.81±19.71[a]	49.16±6.29[a]	74.40±4.61[a]

*Indicates significant differences between positive and negative control groups, while a and b indicate significant differences between positive control group and treated groups;

Table 3: Effects of subacute administration of *T. abut* or *D. macrophylla* extracts on MDA levels in body compartments

Animal group	MDA (µmol/L)			
	Plasma	**Liver**	**Kidney**	**Heart**
NCD	6.83±1.45	3.42±0.13	2.22±0.06	3.70±0.44
HFHSD	20.29±4.06[*a]	53.25±33.47[*a]	39.69±8.86[*a]	25.72±14.67[*a]
HFHS. DM (200mg/kg bw/day)	8.94±2.01[b]	10.62±3.30[b]	4.03±0.99[b]	1.94±0.27[b]
HFHS. DM (400mg/kg bw/day)	14.04±2.67[c]	9.34±2.08[b]	9.06±0.79[c]	7.79±3.20[c]
HFHSD. Statine (10mg/kg bw/day)	13.21±3.05[c]	9.79±1.02[b]	6.99±1.41[c]	8.13±4.13[c]

**Indicates a significant difference between positive and negative control groups, while a, b and c indicate significant differences between positive control groups and the treated groups.*

Table 4: Effect of hydroethanolic extract of *D. macrophylla* berries on Proteins thiol in plasma and tissues

	Thiol protein (µmoles/g of proteins)			
	Plasma	**Liver**	**Kidney**	**Heart**
NCD	471±35.5	150±14.17	300±56.2	253±10.39
HFHSD	201.0±15,64[a*]	124.0±28.48[a]	121.0±14.50[a*]	105.1±17.65[a]
HFHS. DM (200mg/kg bw/day)	216.60±70.35[a]	143.95±77.95[a]	107±19.13[a]	95.05±32.19[a]
HFHS. DM (400mg/kg bw/day)	334.50±38.34[a]	365,6±116.2*[c]	104.70±35.42[a]	78.99±30.83[*b]
HFHSD.Statine (10mg/kg bw/day)	258.10±88.58[a]	115.79±52.08[a]	124.90±51.62[a]	112.0±62.57[a]

*Indicate significant differences between positive and negative control groups, while a, b and c indicate significant differences between positive control groups and the treated groups.

Table 5: Effect of hydroethanolic extract of *D. macrophylla* berries on catalase and nitric oxide levels in blood compartments and heart

Group	**Catalase (**mM H_2O_2 / min/mg protein) **Hemolysates**	**Nitric oxide (µM) Plasma**	**Heart**
NCD	3,45±0.10	6.60± 0.13	6.41± 0.34
HFHSD	1.09±0.32[*a]	19.57±4.96[*a]	18.21±10.79[*a]
HFHS. DM (200mg/kg bw/day)	0.91±0.15[a]	10.93±3.89[*b]	8.09±0.72[*b]
HFHS. DM (400mg/kg bw/day)	0.94±0.06[a]	13.96±1.95[*b]	13.01±1.16[*b]
HFHSD.Statine (10mg/kg bw/day)	1.05±0.45[a]	13.79±2.90[*b]	10.47±3.15[*b]

*Indicate significant differences between positive and negative control groups, while a, b and c indicate significant differences between positive control groups and the treated groups.

Table 6: Effect of hydroethanolic extract of *D. macrophylla* berries on hepatic and renal toxicity markers

Group	**ALAT(U/L)**	**ASAT(U/L)**	**Creatinine (mg/dl)**
NCD	40.61±7.64	20.45± 1.92	1,87±0,30
HFHSD	60.66±18.53[*a]	61.29±6.39[*a]	2.28±0.31
HFHS. DM (200mg/kg bw/day)	72.95±5.72[a]	40.13±8.93[*b]	2.06±0.19
HFHS. DM (400mg/kg bw/day)	68.28±10.72[a]	63.06±14.96[a]	2.32±0.15
HFHSD.Statine (10mg/kg bw/day)	61.63±13.84[a]	57.02±9.40[a]	2.16±0.25

*Indicates significant differences between positive and negative control groups, meanwhile a,b and c indicate significant differences between positive control groups and treated groups.

A decrease in ASAT and an increase in ALAT in treated groups as compared to the HFHS group were observed though not statistically significant. Only DMHE 200mg/kg/bw showed a significant decrease ($p<0.05$) in the level of ASAT. The non significant increase and decrease of ALAT and ASAT activities respectively after treatment of the rats with extracts implied no injury on the liver as well as the heart and other organs which are sources of these enzymes. Also, no significant increase in the level of creatinine was observed between NCD and HFHS groups. This indicates that the HFHS diet as well as DMHE and statin had no effect on the kidneys as indicated by creatinine levels.

CONCLUSION:

This study suggests that the hydroethanolic extract of *Dacryodes macrophylla* possess antioxidant activities which can counteract the weight gain, hyperlipidemia and oxidative damage induced by a high fat-high sucrose diet.

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REFERENCES

1. Haslam W, James P. Obesity. Lancet 2005; 366:1197-1197.
2. World Health Organization: Global Health Risks – Mortality and Burden of Disease Attributable to Selected Major Risks. Geneva: World Health Organization; 2009.
3. Bray GA, Nielsen SJ. Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J. Clin Nutr 2004; 79:537–543.
4. Ford E, De Fronzo RA, Grogan RH. Insulin resistance and endothelial dysfunction. The road map to cardiovascular diseases. Diabetes Metab Res Rev 2005; 22:423-436.
5. Gardner D, Lu L, Lewis TL, Li L. Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. J Biol Chem 2007; 282:36275-36282.
6. Barness LA. Opitz JM. Gilbert-Barness E. Obesity: genetic molecular and environmental aspects. Am Journal of Med Genet 2007; 143A:3016–3034.
7. Howard BV, Ruotolo G. Robbins DC. Obesity and dyslipidemia. Endocrinol Metab Clin North Am 2003; 32:855–867.

8. Bakris G, Stockert J, Molitch M, Zhou Q, Champion A, Bacher P, Sowers J. On behalf of the STAR. Investigators. Risk factor assessment for new onset diabetes: Literature review. Diabetes Obes Metab 2008; Jun 16: Epub ahead of print.
9. Gil-Núñez A. The metabolic syndrome and cerebrovascular disease: suspicion and evidence. Cerebrovasc Dis 2007; 24:S64–75.
10. Ellingsen I. Hjerkinn E. M. Seljeflot I. Arnesen H. and Tonstad S. Consumption of fruit and berries is inversely associated with carotid atherosclerosis in elderly men. Brit J Nutr 2008; 99(3): 674-681.
11. Czernichow S. Vergnaud A.-C. Galan P. Arnaud J. Favier A. Faure H. Huxley R. Hercberg H. Ahluwalia N. Effects of long-term antioxidant supplementation and association of serum antioxidant concentrations with risk of metabolic syndrome in adults. Am J Clin Nutr 2009; 90(2):329-335.
12. Mary Ann Lila. Impact of Bioflavonoids from Berryfruits on Biomarkers of Metabolic Syndrome. Functional Foods in Health and Disease, 2011; 2:13-24.
13. Tsuda T. Regulation of adipocyte function by anthocyanins; possibility of preventing the metabolic syndrome. J. Ag. Food Chem 2008; 56:642-646.
14. Fattorusso V, Ritter, O. Vademecum clinique du traitement au diagnostic. Eds. Masson 1990, Paris, pp 381-387 .
15. Barham D and Trinder P . An improved colour reagent for the determination of blood glucose by the oxidase system. The Analyst 1972; 97:142-145.
16. Jacobs NJ and Vandemark PJ. The purification and properties of the alpha-glycerophosphate-oxidizing enzyme of streptococcus faecalis . Archives of biochemistry and biophysics 1995; 88:250-255.
17. Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clinical Chemistry 1973; 19:476-482.
18. Assmann G. Current diagnosis of hyperlipidemias. Internist (Berl) 1979; 20(11): 559-564.
19. Gutteridge JMC Wikins S. Copper-dependent hydroxyl radical damage to ascorbic acid. Formation of thiobarbituric acid reactive product. FEBS Letters 1982; 337:327-300.
20. Ellman G.L. Tissue sulfahydryl groups. Arch Bioche. Biophys 1959; 82: 70-77.
21. Sinha KA. Colorimetric assay of catalase. Anal Biochem 1972; 47:389-394.

22. Sreejayan Rao MNA. Nitric oxide scavenging by curcuminoids. J Pharmaco 1997;. 49:105-107.
23. Murray RL. Alanine aminotransferase, Aspartate amino transferase and creatinin; In Clin Chem Eds. Kaplan A Peace AL. The C. V. Mosby Co. St Louis Toronto Princeton. (1984).
24. Baena Díez JM, Del Val García JL, Tomàs Pelegrina J, Martínez Martínez JL, Martín Peñacoba R, González Tejón I. et al. Epidemiology of cardiovascular factors. Rev Esp Cardiol 2005; 58:367-73.
25. Unwin N, Roglic G, Whiting D. Type 2 diabetes the challenge of prevention and the WHO Commission on Social Determinants of Health. Diabetes Voice 2010; 55(2):45-47.
26. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. Journal of Clin Invest 2004; 114: 1752–1761.
27. Gregor MF and Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2010; 29: 415–445.
28. Balunas MJ and Kinghorn AD. Drug discovery from medicinal plants. J. Life Sci 2005; 78 431-441.
29. Dziedzic B, Szemraj J, Bartkowiaak J, Walczewska H. Various dietary fats differentially change the gene expression of neuropeptides involved in body weight regulation in rats. J Neuroendocrinol 2007; 19(5):364-373.
30. Kamal A, Amin kamel H, Mohamed A, Eltawab Abd. Protective effect of Garcinia against renal oxidative stress and biomarkers induced by high fat and sucrose diet. Lipid in Health and Disease 2011; 10(6):6 ;10 :(311).
31. Dekker MJ, Su Q, Baker C, Rutledge AC, Adeli K. Fructose: a highly lipogenic nutrient implicated in insulin resistance hepatic steatosis and the metabolic syndrome. Am J Physiol Endocrinol Metab 2010; 299: E685-E694.
32. Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. Life Sci. 2009; 84:705–12.
33. Wouters K, Van Gorp PJ, Bieghs V, Gijbels MJ, Duimel H, Lütjohann D, Kerksiek A, Van Kruchten R, Maeda N, Staels B, van Bilsen M, Shiri-Sverdlov R, Hofker MH. Dietary cholesterol rather than liver steatosis leads to hepatic inflammation in

hyperlipidemic mouse models of nonalcoholic steatohepatitis. Hepatol 2008; 48: 474-486.

34. Yang JS, Lee SJ, Park HW, Cha YS. Effect of genistein with carnitine administration on lipid parameters and obesity in C57Bl/6J mice fed high-fat diet. J Med Food 2006; 9:459-467.
35. Erlund I, Koli R, Alfthan G. Favorable effects of berry consumption on platelet function blood pressure and HDL cholesterol. Am J Clin Nutr 2008; 87(2):323-331.
36. Fukuchi S, Hamaguck K, Seike M, Himeno K, Skata T, Yoshimatsu H. Role of fatty acid composition in the development of metabolic disorder in sucrose induces obese rats. Exp Biol Med 2004; 229(6): 486-493.
37. Törrönen R, Sarkkinen E, Tapola N, Hautaniemi E, Kilpi K, Niskanen L. Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. Br J Nutr 2009;103(8):1094-1097.
38. Noeman A, Saad Hala E, Hamooda Amal A, Baalash. Biochemical study of oxidative stress markers in the liver kidney and heart of high fat diet induced obesity in rats. Diabetol Metab Syndr 2011 3(7):17.
39. Henning SM, Seeram NP, Zhang Y. Strawberry consumption is associated with increased antioxidant capacity in serum. J Med Food 2010; 13(1):116-22.
40. Reil TD, Barnard RJ, Kashyap VS, et al. Diet-induced changes in endothelial-dependent relaxation of the rat aorta. J Surg Res. 1999; 85:96–100.
41. Roberts CK, Barnard RJ, Sindhu RK, et al. Oxidative stress and dysregulation of NAD(P)H oxidase and antioxidant enzymes in diet induced metabolic syndrome. Metabolism. 2006; 55:928–934.
42. Coimbra S, Castro E, Rocha-Pereira P, Rebelo L, Rocha S, Santos-Silva A. The effect of green tea in oxidative stress. Clin Nutr 2006; 25(5):790-796.
43. Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M. Is obesity associated with increased plasma lipid peroxidación and oxidative stress in women. J. ARYA Atheroscler 2007; 2: 189-192.
44. Smith MA, Marley K, Seigler D, Singletary K,Meline B. Bioactive properties of wild blueberry fruits. J. Food Sci. 2000; 65:352-356
45. Higdon J, Frei B. Obesity and oxidative stress: A direct link to CVD? Arterioscler Tromb Vasc Biol 2003; 23: 365-367.

46. Vincent H, Vincent K, Vourguignon C, Braith R. Obesity and postexercise oxidative stress in older women. Med Sci Sports Exer 2005;37: 213–219.
47. Molan AL, Lila MA, Mawson J. Satiety in rats following blueberry extract consumption induced by appetite-suppressing mechanisms unrelated to in vitro or in vivo antioxidant capacity. Food Chem 2008 ; 107:1039-1044.
48. Wasan KM, Najafi S, Wong J, Kwong M. Assessing plasma lipid levels body weight and hepatic and renal toxicity following chronic oral administration of water soluble phytostanol compound FM-VP4 to gerbils. J Pharm Sci 2001;4:228-234.
49. Shimabukuro M, Ohneda M, Unger R. Role of nitric oxide in obesity-induced β cell disease. J Clin Invest 1997; 100:290–295.