ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2010; 1(2): 123 – 130

CASE STUDY

Polyphenols from Dark Chocolate and Their Effects on TheNutritional Status of the Middle Aged Gujarati Jains.

Vanisha Nambiar[1], Shaily Chauhan[1], M. Daniel

[1]*Department of Foods and Nutrition,A WHO Collaborating Center for Health Promotion,* ***Gujrat, India***
[2]***Department of Botany,The Maharaja SayajiRao University of Baroda, Vadodara, Gujarat, India.***

Received 7 April 2010; Accepted 3 May 2010

ABSTRACT

Dark Chocolates (DC) in recent years have gained greater significance & are recommended in the list of functional foods due to the polyphenols (Catechin, Epicatechin and Procyanidins) present in them. The present study is aimed at investigating the polyphenol profile, fat as well as fatty acid profile of dark chocolate available in India and the impact of 50 g DC consumption for a period of 1 month on the nutritional as well as lipid profile of Gujarati Jain subjects. Forty [20 Exp. (M=10, F=10) & 20 Controls (M=10, F=10)] free living healthy Gujarati Jains (30-55yrs of age) were selected for the present study. Pre and post data were elicited on the socio-economic status, anthropometry, dietary pattern, FBS and lipid profile. DC supplementations lead to a reduction of TC (12%), LDL (17.7%) and Non HDL- C (20.0%) ($p< 0.05$) of the Experimental Group as compared to the Control group. However an overall significant increase in the WC (1.9 cm, ($p < 0.05$) & marginal increase in WHR (0.91 vs.0.94) was recorded. These findings indicate that though the polyphenols in dark chocolates had a positive impact on the lipid parameters, DC should be used only as substitutes and not as an addition to the sweets in the daily diets.

Key Words: Dark Chocolate, Polyphenols, Middle Age, Lipid Profile, Dietary Profile.

INTRODUCTION

Chocolates - a functional food is the largest growing snack segment in the U.S and Indian market as its high antioxidant activity has been attributed as a vehicle to lower the risk of coronary heart disease [1]. In India incidences of coronary heart diseases are increasing due to changes in life style and dietary patterns (high energy/fat foods) which have lead to a rise in dyslipedemia [2]. According to the International Cocoa Organization (ICCO) the global chocolate sales estimate were US$74 billion in 2006 which is “significant to increase” over 5 years. In 2009, Swiss chocolate makers bucked the trend with record sales (nearly 185,000 tons), an increase of 2% over 2007, sold domestically and in 140 export markets.

Data on the Western population supports that dark chocolate consumption provides cost-effective heart health care in a delightful way [3]; but there is no Indian study reporting the effect of cocoa or cocoa products including dark chocolates to make recommendations to add the chocolate to your diet as a replacement for a sweet. Eighty percent of the world chocolate market is accounted for by just six transnational companies, including Nestle, Mars and Cadbury, of these; in the present study the Dark chocolate slabs (1kg) were available as gratis from NESTLE (INDIA) Ltd.

In the present study, middle aged Jain subjects (traditionally consuming lower intake of polyphenol rich foods & higher intake of calorie and fat dense foods) were enrolled. Jains have dramatic variation in the consumption pattern: during monsoon, their dietary intake has restriction of polyphenol rich foods like green leafy vegetables whereas they consume a grain and pulse, roots and tuber-less based, traditional dry fruit rich sweets, wedding season food high in fat and sugar in winter [4].

The specific objectives of the present study were

1. To assess the polyphenol, fat and fatty acid profile of dark chocolates.
2. To assess the effect of 50 g dark chocolate supplementation on the nutritional status of the middle aged (30-55yrs) free living Gujarati Jains of urban Vadodara consuming low flavo-noid in the diets for a period of one month.

***Corresponding Author:** Vanisha Naimbar, **Email:** vanishanambiar@gmail.com, **Contact No:** 09327223374

IJPBA, June - July, 2010, Vol. 1, Issue, 2

METHODS AND MATERIAL

Separation & Identification of Polyphenols: The Polyphenol separation included the isolation and identification of (a) Flavonoids (b) Phenolic Acids and (c) Glycoflavones.
Standard procedures [5] were followed for the extraction, isolation and characterization of the individual flavonoids, phenolic acids and glycoflavones from Dark chocolate (*Theobroma cacao)* involving interaction with diagnostic reagents and paper chromatographic separation of compounds and their UV/Visible spectroscopic studies including hypsochromic and bathchromic shifts with reagents such as $AlCl_3$, $AlCl_3$/HCl, NaOMe, NaOAc and NaOAc/H_3PO_3 were followed for the identification of flavonoids and other phenolics. The identities of all the compounds were confirmed by co-chromatography (paper and thin-layer chromatography) with authentic samples. Details of these have been presented in out earlier papers [6].

Total Fat: Fat was estimated from the pre-weighed dark chocolate as per the procedure laid by *NIN (1983)* [7] using the Soxhlet's apparatus and ether as a solvent [8].

Fatty Acid Profile: Fatty acid from chocolate was analyzed by Paul & Southgate (1978) [9] gas chromatographic procedures.

Sampling for clinical trials: Normal subjects (n= 40) who were willing to participate, (30-55yrs of age), followers of Jain religion and with no apparent health complication were selected and divided into experimental and control groups (age and sex matched). [10-15]

Nutritional status data: Pre-post evaluations were elicited on the background information (semi structured Questionnaire), anthropometric measurements (height, weight, waist circumference, hip circumference, waist-hip ratio (WHR)), dietary pattern (24-h dietary recall method and a semi structured questionnaire of dietary habits). For biochemical assessment, overnight fasting and venous blood sample was drawn using disposable needle and syringes. About 5 ml of blood was drawn and collected in centrifuge tubes. The serum was separated and used for various biochemical analysis. These included estimations of FBS and lipid profile [16].

Estimation of Serum Sugar: was done by the enzymatic kit supplied by Glaxo India Limited (GOD/POD method) [17].

Estimation of Triglycerides: The triglycerides in the serum were estimated by the GPO/POD method using the enzymatic kit [18]. **Estimation of Total Cholesterol:** Total cholesterol was estimated using diagnostic kit supplied by Glaxo India Ltd [19]. **Estimation of High Density Lipoprotein (HDL) Cholesterol:** The serum HDL-C was estimated by using the method of Warnick et al.,[20] In serum, LDL-C and VLDL-C was precipated by the addition of phosphotungstic acid and magnesium chloride using the Warnick et al., [20] method. The supernatant was used for HDL-C estimation by the Enzokit as described in total cholesterol estimation. **Estimation of Very Low Density Lipoprotein (VLDL) Cholesterol:** VLDL-C was calculated by dividing triglyceride values by five (TG/5). **Estimation of Low Density Lipoprotein (LDL) Cholesterol:** VLDL-C was calculated by the difference using Friedlewald's formula. LDL-C = TC - (HDL-C + VLDL) as described in our earlier paper (Nambiar et al 2010) [21], before & after one month of 50 g dark chocolate supplementation period.

Dark chocolate supplementation: Dark chocolate slabs obtained as gratis from NESTLE (INDIA) Ltd, were directly weighed and made a portion of 50 g for supplementation.

Statistical analysis: MS Excel was used to assess the means and standard deviation paired "t" test and independent 't" test were used to determine the significant change between the two means. All the tests were considered at significant $p< 0.05$.

RESULTS & DISCUSSION

Polyphenol profile of dark chocolates: The Flavonoids identified by the present study were Catechin, Epicatechin, Cyanidin, Quercetin along with an additional one as Kaempferol; whereas Phenolic acids identified were Vanillic, Syringic, p- coumaric (cis&trans), o- coumaric (Cis&trans), p- OH benzoic acid and Caffeic acid whereas no Glycoflavones were detected (**Table 1 and 2**). Similar reports are obtained by *Arteel et al 1999 and Hammerstone, 2000).*

Table 1: Identified Flavonoids from Dark Chocolate by the Present Study

Class	Compound	Rf Value	Colour Visible	U.V.	Solvent System – Na_2Co_3
Flavonols	Kaempferol	0.35	Light yellow	Yellow	Yellow
	Quercetin	0.25	Yellow	Yellow Brown	Yellow Brown. Dark yellow decomposes
Flavan3ols	(+) Catechin	0.80	–	Light Brown	Light Yellow
	(-) Epicatechin	0.85	–	Light Brown	Light Yellow
	Cyanidin	0.27	Purple pink	Brown	Blue

Table 2: Identified Phenolic Acids by the Present Study

Compounds	Benzene: Acetic acid: Water (6:7:2)	Sodium formate: Formic acid Water (10: 1: 2000)	Colour		
			UV	Nitraaniline	Sulphaaniline
p- OH Benzoic acid	0.22	0.59	Blue	Pink	Yellow
Caffeic acid	0.05	0.55	Colorless	Brown	Brown
Vanillic acid	0.55	0.45	-	Purple	Orange
Syringic acid	0.79	0.51	-	Blue	Red
Cis o–coumaric acid	0.36	0.33	White yellow	Purple	Orange
Trans o–coumaric acid	0.36	0.33	-	Purple	Orange
Cis p–Coumaric acid	0.23	0.72	Dark blue	Blue	Brown pink
Trans p– coumaric acid	0.23	0.72	Dark Blue	Blue	Brown pink

**** 2-5 dihydrobenzoic acid NF-Not found, NA- Not available***

Total Fat: The present study reported the fat content to be 16 g present in 50 g supplemented dark chocolate (**Table 3**) whereas the other chocolate namely the Swiss dark chocolate reported the presence of 17 g of fat as against 19 g fat by *USDA nutrient database 2001*.

Thus 85% of recommended daily allowances for fat for adult men can be obtained only by consuming 50g of DC (*NIN, 2003*).

Table 3: Quantified Fat content in Dark chocolate

Product	Amount of fat present in dark chocolate	
	Present study	Other study
Dark chocolate	16g	17g[1]/19g[2]

1. Semisweet chocolate Converture 2. USDA Nutrient database release July 2001

Fatty acid Profile: The results of fatty acid profile obtained by gas chromatography are shown in (**Table 4**). Fatty acids were eluted in 42.6 minutes; stearic acid was identified as the main peak. were consistent with the ones reported by *USDA Nutrient database 2001* namely SAFA - Stearic acid - **36.9%** vs. 35%, Palmitic acid – **25.7 %** vs. 25% MUFA - Oleic acid – **33.3%** vs. 30% PUFA - Linoleic acid – **2.7%** vs. 3%, Linolenic acid – **1.1%** vs. 0.3%.

Stearic acid is a SAFA – the type that would normally be expected to raise the blood cholesterol levels in the body. However for various reasons, the effect of stearic acid in vivo has not been clearly understood *(Steinberg et al 2003)*. Oleic acid, the same fat in olive oil is a MUFA and has been shown to benefit heart health *(Steinberg et al 2003).*

Table 4: Distribution of Fatty Acid of Chocolate Fat

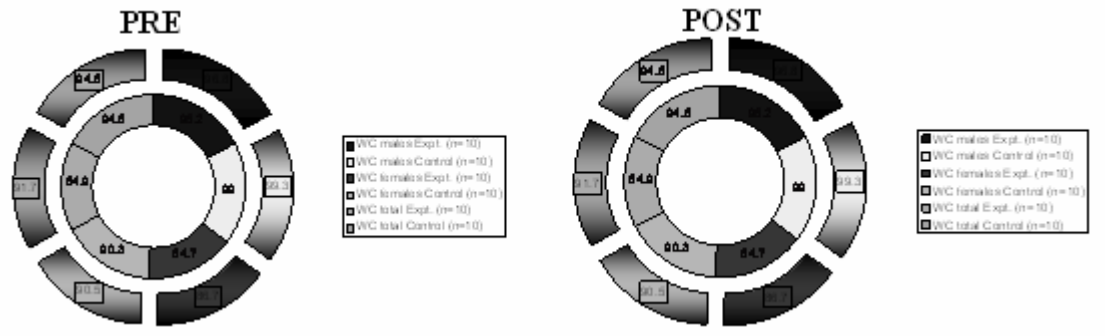
Retention Time (min.)	Type of Carbon Chain	Fatty Acid	No. of Carbon Atom	Conc. Present Study (%)	Conc. Reported Study (%)
31.11	SAFA	Palmitic	16:0	25.44	25
37.00	SAFA	Stearic	18:0	33.41	35
37.97	MUFA	Oleic	18:1	37.02	30 – 35
39.73	PUFA	Linoleic	18:2	2.73	3
42.6	PUFA	Linolenic	18:3	1.18	0.3

Clinical trial results: Impact of Dark chocolate supplementation on the nutritional status of middle aged Jains with no reported health complications are discussed under following sections:

SES: The socio demographic attributes reveal that the subjects being the followers of Jainism did not have any habit of smoking, drinking alcohol or consuming non veg, food but restrict their dietary intake of protective & polyphenol rich foods like

roots, and tubers, Green Leafy Vegetables exclusively for 4 months pose them at a higher risk of any disease complication. The mean height and weight was 1.61cms and 67.2 Kg respectively. All the subjects were free from any reported disease condition.

Fig: Waist Circumference and Waist Hip Ratio of the Subjects before & After Dark Chocolate Supplementation



Anthropometric Measurements: Post supplementation results on Anthropometric measurements shown in **Table 5** reveals a non significant change in BMI (24.1± 4.1 to 24.1± 4.3), The mean WC values of the subjects increased on DC supplementation by 1.6 and 2.0 cm in the Exp. males and females leading to an overall significant increase of 1.9 cms in total. However there was a 20% & 10% decrease in the normal male and female subjects respectively as per the WC which shifted to overweight / obese category.

When the subjects were categorized as per the WHR a marginal increase in overweight/obese subjects was seen. It was found to be 0.91 and 0.92 of the Exp. and Control group, which after the supplementation increased to 0.94 in both the groups. Thus the subjects were found to be at risk for several disease conditions due to their anthropometric measurements being above their normal values.

Table 5: Anthropometric Measurements of the Subjects Before and After Supplementation

Measurements	Males (n=20)		Females (n=20)		Total (n=40)	
	Expt. (n=10)	Control (n=10)	Expt. (n=10)	Control (n=10)	Expt. (n=10)	Control (n=10)
Age (years)	44.6±5.96	36.5±5.09	45.0±7.04	44.6±5.69	44.8±6.5	40.4±5.39
Height (cm)	1.67±0.07	1.54±0.03	1.68±0.07	1.55±0.03	1.67±0.07	1.54±0.04
Weight (kg)						
Pre	69.0±11.3	76.0±18.3	56.9±10.1	66.9±9.2	62.9±10.8	71.4±13.7
Post	70.4±11.6	75.7±18.5	57.9±10.4	67.4±9.3	64.1±11.0	71.5±13.9
t value	2.08	0.60	2.64**	0.66	0.60	0.21
BMI (kg/m^2)						
Pre	24.3±4.8	26.7±5.6	23.9±3.8	27.6±3.7	24.1±4.3	27.1±4.6
Post	24.4±4.2	26.5±5.6	24.2±4.1	27.4±3.4	24.3±4.1	26.9±4.5
t value	1.80	0.88	1.49	0.47	1.27	0.72
WC (cms)						
Pre	95.2±8.6	99.0±12.3	84.7±11.7	90.3±10.4	84.9±10.1	94.6±11.3
Post	96.8±9.7	99.3±12.7	86.7±10.1	90.5±9.3	91.7±9.9	94.6±11.3
t value	1.56	0.47	1.9	0.4	2.39**	0.61
WHR						
Pre	0.98±0.06	1.00±0.99	0.85±0.07	0.84±0.07	0.91±0.06	0.92±0.53
Post	1.00±0.07	1.04±0.08	0.89±0.09	0.84±0.07	0.94±0.08	0.94±0.07
t value	0.84	1.79	1.22	0.08	1.51	1.05

***** Significantly different at p<0.05.***

Dietary Assessments:

The chocolates were simply added to the subject's diet with the restriction in the consumption of flavonoid rich foods like onions, garlic and tea. The polyphenol intake showed a significant difference ($p<0.05$) in both males and females before and after supplementation (males: 214.7 vs. 601.7 mg/day), (females: 185.5 vs. 565.0 mg/day) than compared to the ideal intake of <1000 mg/day.

Biochemical Profile:

The biochemical indices of the subjects under the study population are represented in the **Table 6**. Overall the present study resulted in significant reduction in TC (12%), LDL – C (17.7%) & Non HDL–C (20.0%) after 1 month dark chocolate supplementation.

Table 6: Biochemical Indices of the Subjects Before and After Supplementation

	Normal Levels	Males (n=20)		Females (n=20)		Total (n=40)	
		Expt. (n=10)	Control (n=10)	Expt. (n=10)	Control (n=10)	Expt. (n=10)	Control (n=10)
TC	< 200						
Pre		188.7±26.7	176.6±33.9	177.0±21.7	179.1±33.4	182.8±21.8	177.8±33.6
Post		161.7±22.0	164.2±34.3	155.9±18.4	187.0±15.0	158.8±20.2	175.6±24.6
t value		1.09	0.76	2.76**	2.06	5.28**	0.35
TG	< 130						
Pre		118.4±35.2	97.2±19.8	101.3±22.5	110.9±28.2	109.8±28.8	104.0±24.0
Post		111.0±40.2	93.8±16.8	95.3±46.6	99.3±18.8	103.1±43.4	96.5±17.8
t value		1.02	0.82	0.45	1.67	0.91	1.86
HDL	> 60						
Pre		36.2±7.5	42.2±8.23	37.6±7.5	43.2±8.2	36.9±7.51	42.7±8.2
Post		39.6±12.6	43.2±9.16	38.3±9.3	43.5±7.6	38.9±10.9	43.3±8.3
t value		0.07	0.07	0.23	0.72	0.88	0.35
LDL	< 100						
Pre		129.3±25.9	114.9±36.2	102.0±37.1	113.7±35.3	115.6±31.5	114.3±35.7
Post		100.3±23.4	102.2±38.0	95.5±29.7	123.6±18.0	97.9±26.5	112.9±28.0
t value		4.57**	1.87	0.53	0.85	2.49**	0.19
VLDL	Upto 34						
Pre		23.0±7.2	19.4±3.9	20.2±4.5	22.1±5.6	21.6±5.85	20.7±4.8
Post		22.0±8.04	18.7±3.6	19.0±9.3	19.8±3.7	20.5±8.6	19.2±3.6
t value		0.63	0.81	0.44	1.68	0.71	1.86
FBG	< 100						
Pre		69.7±17.7	57.7±8.8	61.0±12.4	58.2±6.9	65.3±15.0	57.9±7.8
Post		70.0±28.2	54.6±11.9	65.1±24.0	61.5±8.6	67.5±26.1	58.0±10.2
t value		0.03	1.75	0.71	1.17	0.45	0.04
Non HDL	< 130						
Pre		152.5±11.2	134.4±25.6	139.4±14.2	135.7±25.2	145.9±14.2	135.1±25.4
Post		122.1±9.4	121.0±25.1	117.6±9.1	143.5±7.4	119.9±9.3	132.3±16.3
t value		5.35**	1.97	2.27**	0.61	4.72**	0.39

***** Significantly different at p<0.05.***

Total Cholesterol (TC): The overall total cholesterol values of the experimental group reveal a significant decrease ($p<0.05$) of 24mg as compared to 2 mg decrease in the Controls.

This is at par with the Feeding studies conducted at Pennsylvania State University that chocolate did not elevate total and LDL concentration in blood *(Etherson et al 1994).* Stearic acid found in chocolate has been shown to be benign in raising serum cholesterol levels *(Connor et al 2000).* It has been shown that stearic acids are converted to oleic acid in the liver which is unsaturated. Thus do not contribute to hypercholesterolemia *(Etherson et al 1994).*

Triglyceride (TG) & High Density Lipoprotein (HDL): The mean levels show no significant change in the TG as well as HDL-C values in the Experimental & Control group.

Low density lipoprotein (LDL-C): There was a significant reduction in LDL levels of the Experimental male subjects (29 mg) as compared to Control males (12mg). Overall the LDL values significantly decreased to 18 mg (from 115.6mg to 97.9 mg) in the Experimental group as compared to only 2mg change in the Control group.

Very low density lipoprotein (VLDL-C): VLDL-C values showed similar results like TG and HDL.(**Table 7**) The mean values before and after supplementation of the Exp. males were (23.0 to 22.0 mg/dl) and females being (22.1 to 19.8 mg/dl) comparable with the controls.

Table 7: Atherogenic Indices of the Subjects Before and After Supplementation

	Normal Levels	Males (n=20)		Females (n=20)		Total (n=40)	
		Expt. (n=10)	Control (n=10)	Expt. (n=10)	Control (n=10)	Expt. (n=10)	Control (n=10)
TC/H	3.3 -3.6						
Pre		5.21±3.5	4.18±4.1	4.7±2.8	4.14±4.05	4.9±3.15	4.16±4.07
Post		4.08±1.7	3.8±3.7	4.0±1.97	4.29±1.97	4.04±1.83	4.0±2.8
t value		1.95	3.95**	1.33	0.09	2.29**	0.50
L/H	2.5-3.5						
Pre		3.5±3.4	2.7±4.3	2.71±4.94	2.63±4.2	3.1±4.17	2.6±4.2
Post		2.5±1.85	2.36±4.14	2.49±3.19	2.84±2.36	2.51±2.5	2.5±3.2
t value		1.70	0.53	0.93	1.24	1.24	1.02

***** Significantly different at p<0.05. Reference- New ATP III guidelines 2001, Indo Asian guidelines***

When the TG levels go beyond > 150mg/dl usually then VLDL levels increases to more than 30 mg/dl. *(New ATP III Guidelines, May 2001).* But in the present study due to dark chocolate supplementation and the normal TG levels of the subjects the VLDL resulted into normal levels.

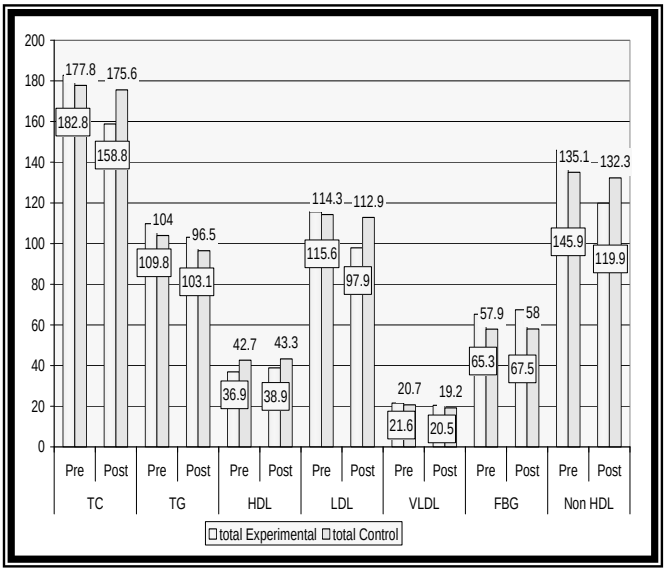
Fasting Blood Glucose (FBG): The mean FBG values of the Exp. & Control group was found to be consistent before and after supplementation. Their mean levels being (65.3 vs.67.5 mg/dl) whereas in the Controls being (57.9 vs. 58.0 mg/dl) respectively. These values also reflect the long lasting period of 12 hrs. between the dinner which is taken before sunset as per Jain customs.

Non HDL-C: There was 23.4% reduction in Non HDL-C values in the Exp. males and 16.8% decrease in Exp. females with an overall 20.0% significant reduction in the subjects of Exp. group as compared to 2.2% in total Controls.

Atherogenic Indices: The atherogenic indices as shown in **Table 7** also clearly indicate that there is a positive effect of Dark chocolate supplementation in both the male and females as a significant lower values of TC/H are seen in the Exp. group (4.9 vs.4.04) vs. Control group (4.16 vs. 4.0) with a difference of 0.86 & 0.16 respectively whereas no significant changes were seen in the L/H & TC/L values.

The results of the present biochemical profile as shown in **Fig 2** clearly supports that the flavonoids present in DC have a cardio protective effect, and this is supported by the positive significant change in the lipid parameters along with the significant decrease in TC/H and marginal decrease in TC/L & L/H ratios, an atherogenic indices.

Fig 2: Graph Showing Biochemical Indices of the Total Subjects Before & After Dark Chocolate Supplementation



DISCUSSION

Chocolates have replaced most of the traditional sweets during celebrations & it is utmost important to study the effects of the same on Indians, especially populations consuming high fat and have a low fruit and vegetable intake. But ironically, countries with fast-growing middle-classes and large populations like China and India are regarded as potentially huge international trade markets for chocolate manufacturers and Chocolates make up India's half confectionery market, with hard-boiled candies and toffees making up nearly 40 percent. These days, a typical sweet 50 g portion of Indian sweet (a high morsel of fat & calorie) is substituted with a 50 g piece of chocolate. A single piece of chocolate is considered to be 50 g produced by 200 cocoa beans *(Marcus 2001).* The higher the cacao content in chocolate, the higher the amount of the pharmacologic chemicals in the chocolate (*Etherson et al, 1994*). It has been reported that chocolates without cooking as the raw slabs has higher antioxidant activity then the conventional ones *(Gu et al 2006).*

There is a strong linear correlation between Non Fat Cocoa Solids & Oxygen Radical antioxidant

Capacity *(Miller 2006, Nambiar and Chauhan, 2006). Engler (2004)* investigated effect of flavonoid rich Dark Chocolate on blood lipids & B.P in healthy subjects in age group of 21-55yrs who had to consume 46g dark chocolate. Various Dark Chocolate studies (*Mathur et al 2002, Scramm et al 2001, Engler et al 2004, Wang et al 2001*) have been conducted on middle age population; being vulnerable to have high lipid & fasting glucose levels due to various reasons like poor eating habits, stress, sedentary lifestyle *(Park et al 2006).*

Tracing the genesis of Chocolates, it comes from an agricultural product and consumption of smaller portion serves beneficial *(Steinberg et al 2003, chocolate.org).* Cacao contains polyphenols namely flavan3ol-catechin, epicatechin and oligomeric procyanidins (plant-based compounds) *(Liu et al 2000 & Joshipura et al 1999)* which have been reported to have highest flavonoid antioxidant quantity – quality activity than the ones found in fruits, vegetables, red wines and black tea *[Oxygen Radical Anti-oxidant Capacity (ORAC) data-Chocolate Information Centre 2001].* These may help reduce oxidation of LDL cholesterol (low-density lipoprotein) and may help modulate platelet activity, both of which contribute to cardiovascular disease *(Rein et al 2000, Paglieroni et al 2000, Larson 2001).*

Even though a bar of chocolate exhibits strong antioxidant activity, the health benefits are still controversial because relatively large amounts of saturated fats are present. However, a cup of hot cocoa has a much lower level of saturated fats (0.3 g per serving) than a bar of chocolate (8 g per 40 g bar).

This is the first Indian study reporting the effect of cocoa or cocoa products including dark chocolates.

Thus the results of the present study support the concept of favorable effects of lipid health of the subjects. However higher BMI, WC and WHR indicates that though chocolates offer strong antioxidant support in a delightful way it has to be consumed There is a need of modifying the Nutritional Components of the supplemented dark chocolate in terms of decreasing the fat & CHO content, increasing the calcium content which may drive down the stearic acid content of these chocolates in vivo, increasing the cocoa content and make it more concentrated as it contains essential trace elements and nutrients such as iron, calcium, potassium and excellent source of magnesium and vitamins which are beneficial for the cardiovascular system and hypertension.

All these need to be encouraged so that we can more intelligently select a protocol for testing the validity of the hypothesis in humans.

REFERENCES

1. Arteel GE, Sies H. Protection against peroxynitrite by cocoa polyphenol oligomers 1999; FEBS Lett. 462:167–170.
2. Connor WE. Harbingers of Coronary Heart Disease; Dietary Saturated Fatty Acids and Cholestrol. Is Chocolate benign because of its stearic acid content? Am. J. Clin Nutr. 1999; 70–95.
3. Daniel M. Polyphenols of Indian vegetables. Curr. Sci. 1989; 58(23):1332–1334.
4. Engler MB, Engler MM, Chung Y, et al. Flavonoid Rich Dark Chocolate improves endothelial functions and increase plasma epicatechin concentration in healthy adults. Jr. of Nutr 2004; 23: 197–204.
5. Etherson.K PM, Derr JA, Mitchell DC. A Milk Chocolate bar/day substituted for high CHO snack increases high density lipoprotein cholesterol in young men on NCEP/AHA stepore diet. Am Jr. Clin Nutr 1994; 60:1037S–1042S.
6. Gu L, Wu.X, et al. Procyanidin and Catechin Contents and Antioxidant Capacity of Cocoa and Chocolate Products. J. Agric. Food Chem. 2006, 54: 4057-4061.
7. Hammerstone JF, Lazarus SA, Schmitz HH. Procyanidin content and variation in some commonly consumed foods. J Nutr 2000; 130:2086S–92S.
8. Joshipura KJ, Ascherio A, Manson JE, Stampfer M, Rimmeb, Speizer F. Fruit & Vegetable intake in relation to risk of ischemic stroke JAMA 1999; 282:1233–1239.
9. Liu S, Lee IM, Ajani U, Cole SR, Buring JE, Manson JE. Intake of vegetables rich in carotenoids and risk of coronary heart disease in men. The physicians' Health Study. Int. Jr. Epidemiology 2000; 30:130.
10. Mabry TJ, Markham KR. The systematic identification of flavonoids, Springer-Verlag 1970; Berlin.
11. Mathur S, Devaraj S. Cocoa Products decrease LDL oxidative susceptibility but donot affect biomarkers of inflammation in humans. Jr. Nutr 2002; 32:3663–3667.

12. Miller K, Stuart T et al (2006). Antioxidant Activity and Polyphenol and Procyanidin Contents of Selected Commercially Available Cocoa-Containing and Chocolate Products in the United States. J. Agric. Food Chem. 2006, 54, 4062-4068
13. Nambiar VS, Guin P, Parnami S, Daniel M. Impact of antioxidants from drumstick leaves on the lipid profile of hyperlipidemics, Journal of Herbal Medicines and Toxicology 2010; 4 (1): 165-172.
14. Nambiar VS, Daniel M, Guin P. Characterization of polyphenols from coriander leaves (C*oriandrum sativum*), red amaranthus (A. *paniculatus*) and green amaranthus (A. *frumentaceus*) using paper chromatography and their health implications. Journal of Herbal Medicines and Toxicology 2010; 4 (1): 173-177.
15. Nambiar VS, Guin P. Prevalence of hyperglycemia and hyperlipidemia among the middle aged and elderly population in a University setup. **I**ndian Journal of Gerontology 2007; 21(1): 30-43.
16. Nambiar VS, Chauhan S. Health effects of Chocolates. Research Reach**.** Journal of Home Science 2006; 5 (2): 1-13.
17. Nambiar VS, Mehta R, Daniel M. Polyphenol Content of Three Indian Green Leafy Vegetables. Journal of Food Science and Technology 2005; 42 (6): 312-315.
18. Nambiar VS, Seshadri S. Dietary patterns and its relation to relation to disease profile in postmenopausal women (part II**).** Indian Journal of Gerontology 2004; 18 (8): 73-84.
19. Ed Gopalan et al. Nutritive value of Indian foods. National Institute of Nutrition/ ICMR 2003; Hyderabad.
20. New ATPIII. The third report of the expert panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2002.
21. Pearson DA, Schmitz HH, Lazarus SA, Keen CL. Inhibition of in vitro low–density lipoprotein oxidation by oligomeric procyanidins present in chocolate and cocas. Methods Enzymol 2001; 335:350–60.
22. Schramm DD, Wang JF, Holt RR. Chocolate procyanidins decrease the leukocriene–prostacyclin ratio in humans aortic endothelial cells. Am J Clin Nutr 2001;37:36–40.
23. Shah J. To study the effect of cereal pulse mix supplementation on lipid profile of hyperlipidemic subjects. M.Sc. Dissertation, Department of Foods & Nutrition, Faculty of Home Science, The M. S. University of Baroda 1995, Vadodara.
24. Steinberg FM, Bearden M, Keen C. Cocoa and Chocolate flavonoids: Implciation for cardiovascular health. Jr. AM. Diet Asso. 2003; 103:215–223.
25. USDA – United States. Department of Agriculture July Release 2001
26. Wang Y, Vinson J, Etherton TD, Proch J, Lazarus SA, Kris–Etherton PM. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. Am. J. Clin. Nutr 2001; 74:596–602.