Original Research Article

EVALUATION OF INSULIN, MDA AND BLOOD PRESSURE IN MALE OBESE INDIVIDUALS IN NNEWI AND SUBSEQUENT EFFECT OF GREEN TEA SUPPLEMENTATION.

ABSTRACT

Background Obesity is a major public health issues worldwide, contributing to increase in cardiovascular disease, diabetes, insulin resistance and oxidative stress. This is due to sedentary lifestyle, poor dieting and low antioxidant supplement consumption example green tea. The objective of this study is to evaluate the level of fasting blood sugar, insulin, insulin resistance blood pressure and MDA in obese subject and subsequent effect of green tea at 6weeks and 12weeks supplementation.

Method: It involves cross sectional and interventional study. In cross sectional study, 88 obese subjects (46 class I and 42 class II obese) and 50 normal weight subject (control) were recruited. Fasting blood samples were collected and analyzed for the different parameters. HOMA-IR was calculated. In interventional study, 20 male obese were randomly selected and were given 200mls of commercially prepared green tea (2 tea bag dissolved in 200ml of boiled water) once daily for 12weeks. Their fasting blood samples were collected before the intervention (baseline), at 6weeks and after 12weeks intervention and were later analyzed by standard method Enzyme Linked immunoassay and colorimeteric method. Statistics was done with statistical tool SPSS version 21.

Result: There were significant increases in mean level of HOMA-IR, systolic and diastolic blood pressure, fasting plasma glucose and insulin in obese subjects (class II and class I obese) when compared with control group ($P<0.05$), likewise in Class II obese when compared with Class I obese ($P<0.05$) while in case of MDA, there was significant increase only in Class II obese subjects when compared with the normal weight subject($P<0.05$).Green tea supplementation significantly reduced the mean level of MDA, fasting plasma glucose, weight, HOMA-IR and blood pressure at 12weeks intervention while only Insulin and waist circumference were significantly reduced at 6weeks and 12weeks intervention. Conclusion: In conclusion, increase in severity of obesity exposes the individual to more complications and health related problems of obesity especially hypertension, oxidative stress and diabetes mellitus and subsequent supplementation with green tea for 12weeks can ameriorate these disorders caused by obesity.

Keywords: Obesity, green tea, blood pressure, insulin resistance, oxidative stress

1. INTRODUCTION

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health. Recent studies have shown that obesity-associated risk factors depend not on excess body weight *per se*, but rather on the regional distribution of the

excess body fat. In light of this, it is now well recognized that abdominal fat is a significant risk factor for obesity-associated diseases; in fact, visceral fat accumulation stimulates pro-oxidant and proinflammatory states [1]. In obesity, modulation of metabolic pathways plays critical roles in the pathogenesis of many diseases [2]. There is a strong positive association between obesity and type II diabetes, dyslipidaemia, cardiovascular disease, and hypertension [3]. Hence, creation of appropriate strategies to reduce weight, insulin resistance, oxidative stress and to increase total antioxidant capacity in obese, have been the focus of this study.

Recent studies on humans show that green tea has many health benefits including reduced risk of cardiovascular disease and some cancers, anti-effects on blood pressure, weight loss, antivirus and antibacterial activities, anti-mutagenic, anti-inflammatory and decrease insulin resistance [4]. Green tea contain appreciable amounts of phytochemicals especially catechins that further comprised of different chemical moieties that include epigallocatechin-3-gallate (EGCG), epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC). Amongst these, EGCG is present in higher amounts and considered to be an effective antioxidant. Because of the high rate of green tea consumption in worldwide populations, its small quantity could have a large public health impact on individual basis. In the recent era, diet based therapy has been revitalized globally and people are adopting the approach of using natural materials as an intervention against various ailments [5]. Keeping in view the health challenges associated with obesity, limited research and controversial findings on effect of green tea on ameriorating blood pressures, oxidative stress and diabetes, this study aim at evaluating these parameters in obesity and subsequent effect of green tea supplementation.

2. METHODS

2.1 Study Design: The research was carried out at Nnewi, Anambra state, Nigeria and its biochemical analysis was done at Nnamdi Azikiwe Teaching Hospital (NAUTH) Nnewi, Anambra State, Eastern Nigeria. This hospital was chosen because they have competent personnel (Medical laboratory scientist) and equipment. In cross sectional study, 88 obese subjects (46 class I and 42 class II obese)

and 50 normal weight subject (control) were recruited. In interventional study, 20 male obese were randomly selected and were given green tea.

Source of green tea: The green tea was obtained from Lipton Company (Unilever Ghana Ltd (GH) brand and was of the same brand and batch number 16252. NAFDAC Reg. NO: B1-8866. Phytochemical analysis on the green tea was performed on a BUCK M910 gas chromatography equipped with a flame ionization detector according to Kelly D; Nelson R; [6] to note the concentration of active ingredients (phenol) present.

Preparation of green tea: Two (2) green tea bags, each weighing 1.6g were dissolved in 200ml boiled water. This was left to dissolve for 5mins before consumption. They took the green tea once daily for 12weeks (3months).

2.2 Inclusion criteria and Exclusion criteria:

Subjects recruited were between the age of 29 and 47yrs with body mass index 30 - 35 Kg/m^2 (for Class I obese), 35 – 40 (class II obese), 19-24.9 Kg/m^2 for non-obese (controls). Apparently healthy individual were used, they were not on any medications for diabetes, hypertension, other CVD. Subject on alcohol, cigarette, children, adolescents, morbid obese (BMI above 41Kg/m^2), bedridden, physically challenged, and subject above 50 years were excluded from the study.

2.3 Ethical approval and informed consent:

Ethical approval was sought and obtained from the Research Ethics Committee of the Nnamdi Azikiwe University Teaching hospital (RECNAUTH) Nnewi, Anambra state with reference NAUTH/CS/66/VOL10/2017/010. The participants were informed about the study designs; their written informed consent was obtained before they were recruited.

2.4 Data Collection Procedure: Subjects who indicated interest in the study, following discussion at business areas, church, offices, recreation outfits, and restaurant were given detailed designed questionnaire to fill. It contains information about the subject's demographic data, anthropometric

measurements, biochemical details, and clinical parameter (lifestyle and dietary habits, medical and family history).

2.5 Anthropometric measurements: The weights of the subjects were evaluated with scale (Gulfex Medical and Scientific, England)). Weight was read off in kilograms (kg) and recorded to the nearest 0.1kg value. The subjects' height was recorded in meters using a height scale calibrated in centimeters, and the reading taken to the nearest 0.1cm value. As a measure of generalized obesity, each adult participant's BMI was computed by dividing the weight in kilograms, by the square of the height in meters (kg/m^2) To determine abdominal obesity, measurement of the waist circumference (WC) was taken using a stretch-resistant tape (HTS, China). Blood pressure (BP) systolic and diastolic pressure readings were taken from the participant's left arm using sphygmomanometer (Omron Medical, United Kingdom), after being seated for ten minutes. The reading was taken in the morning to the nearest mmHg.

2.6 Sample collection, Storage and Analysis

5mls of blood sample was collected from fasting subjects between 8 and 10am using standard procedure as described by Lewis et al., (2006). 1ml of whole blood was dispensed into fluoride oxalate bottle and the plasma separated for glucose analysis while the remaining 4mls of whole blood was dispensed into plain bottle and allowed to clot, retracted and spun at 3000rpm for 10minutes. Thereafter, the serum was separated into two aliquots and stored. Plasma glucose was analyzed immediately while serum if not assayed immediately was stored at -20°C not more than 2weeks before analyses. For the cross- sectional study, one point blood sample was collected from each participant both for normal weight and test subjects while in intervention study, three points sample was collected from each test subjects: baseline, 6weeks and 12weeks following green tea supplementation. Glucose was assayed colorimetrically using Glucose oxidase method of Trinder, (1969) [8]. MDA level was determined by the colorimetric method of Gutteridge and Wilkins, [9]. The serum insulin level was estimated based on solid phase enzyme linked immunosorbent assay (ELISA) method using ACUBIND kit and mindray (MR- 96A) ELISA machine. Insulin Resistance

(IR), was assessed by homoeostasis model assessment–insulin-resistance index (HOMA–IR), according to the following formulas: 'fasting insulin value (mU/L) × fasting blood sugar level (mmol/L) / 22.5' [10], values exceeding 2.25 would denote insulin resistance. Quality control was ensured by using pooled control sera from apparently healthy individual and commercially purchased control (Randox (USA) Control level 1.

2.7 Statistical analyses

Statistical analyses were performed using statistical package for social sciences (SPSS) software version 21.0 software. The variables were expressed as mean ± SD. A preliminary comparison of differences between obese Class I , Class II, and non-obese (control), was assessed using Analysis of Variance (ANOVA) while Post Hoc was used for inter-group variability. Paired t-test was used to assess the mean difference between two related variables and level of significant was considered at $P<0.05$.

3.0 RESULTS

3.1 Anthropometric measurement in Obese (Class II and Class I) and normal weight groups (control)

The test groups were age matched with the control group, therefore there was no significant difference in the mean age across the groups ($P>0.05$). (Class II; 38.2±5.26, Class I ; 38.95±5.69, normal weight group (control) 36.6 ±5.1). There were significant increases in the mean level of weight, waist circumference, W/H ratio, height, BMI in obese subjects (class II and class I obese) when compared with control group ($P<0.05$), likewise in Class II obese when compared with Class I obese ($P<0.05$) except height which did not decrease significantly.

Table 1 Anthropometric measurement in Obese (Class II and Class I) and non-obese groups (control) MEAN ± SD

PARAMETER	CLASSII Obesity	CLASS I Obesity	CONTROL (normal weight)	Fvalue	POST HOC Pvalue	B/C	A/C	A/B
AGE (yrs.)	38.2±5.26	38.95±5.69	36.6 ±5.1	2.6	0.075	0.087	0.435	1.000
HEIGHT (m)	1.7 ± 0.07	1.72 ±.071	1.75 ±.07	7.6	.001*	.045*	.000*	0.624
WEIGHT(kg)	109±8.35	97 ±8.45	69.7 ±4.6	423.6	.000*	.000*	.001*	.000*
BMI (kg/m^2)	38.2±1.06	32.9 ±1.06	22.7 ±1.1	2542	.000*	.000*	.000*	.000*
WAIST(cm)	114± 7.28	106 ±5.1	85 ± 9.0	206	.000*	.000*	.000*	.000*
HIP (cm)	111.9±9.7	111.8 ±4.6	96 ± 9.7	59.5	0.000*	0.000*	0.000*	1.000
W/H RATIO	1.03±.059	0.95 ±.05	0.89 ±.07	59.5	0.000*	0.000*	0.000*	0.000*

*KEY : A represents class ii obesity, B represents class i obesity, C represents control. BMI = Body mass index, Key * = Results compared are significantly different at P-value < 0.05 (P < 0.05).*

In table 2, the mean level of fasting plasma glucose, insulin, HOMA-IR, systolic and diastolic pressure increased significantly in obese group (class II and class I) when compared with their control likewise in Class II obese when compared with Class I obese (P<0.05) while in case of MDA, significant increase was found only in Class II obese subjects when compared with the normal weight subjects.

Table 2 Mean Fasting plasma glucose, fasting blood insulin, HOMA-IR and blood pressure in obese subject (Class II and Class I) and non-obese groups (control) MEAN ± SD

						POST HOC		
PARAMETER	Class II Obesity	CLASS I Obesity	Normal weight	Fvalue	Pvalue	BvsC	AvsC	BvsC
SBP (mm/Hg)	136.9±8.0	130.9±14	123.7±7.8	20.5	.000*	0.012*	0.000*	0.026*
FPG (mmol/L)	6.04 ±.77	5.59 ±.88	5.12 ±.74	16	0.000*	0.035*	0.000*	0.012*
INSULIN (μIU/ml)	7.7 ±2.6	6.3 ±2.2	4.7 ±1.4	26	0.001*	0.019*	0.000*	0.014*
HOMA-IR	2.1 ±.75	1.6 ±.61	1.05 ±.32	39.5	0.000*	0.001*	0.000*	0.000*
DBP(mm/Hg)	95.2±7.1	88 ±9.8	82.9 ±8.9	23	0.000*	0.001*	.000*	0.012*
MDA(nmol/ml)	3.94±1.27	3.72 ±.91	3.30 ±.87	5.1	.007*	0.122	.008*	0.985

Key * = Results compared are significantly different at P-value < 0.05 (P < 0.05). *KEY : A represents class ii obesity, B represents class i obesity, C represents control.* ***FPG****= Fasting Plasma Glucose,* ***HOMA-IR*** *Homeostatic Model Assessment-Insulin Resistance,* ***MDA*** *malondealdehyde,* ***SBP****, systolic Blood Pressure,* ***DBP*** *Diastolic Blood Pressure.*

In table 3, significant weight loss was observed only after 12 weeks green tea supplementation when compared with baseline and also in 12weeks when compared with 6weeks supplementation(P<0.05) unlike waist circumference which reduced significantly after 6 and 12wks intervention when compared with baseline (P <0.05). Furthermore on 12weeks supplementation, there were significant decrease in systolic blood pressure, fasting plasma glucose, fasting blood insulin, MDA, homeostatic model assessment - Insulin resistance (HOMA-IR) (P <0.05) when compared with their baseline, however FPG, HOMA-IR, MDA and systolic pressure did not significantly decrease after the first

6weeks intervention (P>0.05) Furthermore diastolic blood pressure did not reduce significantly throughout the 12weeks supplementation (P>0.05).

Table 3 Mean level of blood pressure, Fasting Blood Glucose, Fasting Blood Insulin, MDA and HOMA-IR at different stages of green tea supplementation

PARAMETERS	BASELINE	6WEEKS	12WEEK	POST HOC		
N= 20	(A)	(B)	(C)	A vs B	A vs C	B vs C
MDA (nmol/L)	3.95±.66	3.83±.97	3.31±.88	0.693	0.018*	0.071
Waist circu. (cm)	112.6±8.9	112.2±9.2	111.9±9.5	0.016*	0.006*	0.297
SBP (mm/Hg)	133.6±8.8	133.5±8.9	132.9±8.6	0.614	0.031*	0.017*
DBP (mm/Hg)	93.5±7.6	93.35±7.84	93.1±7.85	0.614	0.104	0.204
FPG (mmol/L)	5.6±.83	5.4±.77	5.3±.75	0.089	0.003*	0.031*
FBI (uIU/L)	7.9±1.2	6.0±1.6	5.14±.99	0.000*	0.001*	0.065
HOMA-IR	2.0±.74	2.2±.61	1.6±.47	0.316	0.039*	0.006*

Key * = Results compared are significantly different at P-value < 0.05 (P < 0.05).

FPG*= Fasting Plasma Glucose,* ***HOMA-IR*** *Homeostatic Model Assessment-Insulin Resistance,* ***MDA*** *malondealdehyde,* ***SBP****, systolic Blood Pressure,* ***DBP*** *Diastolic Blood Pressure*

Disscussion

Obesity is becoming one of the most prevalent health concerns among all populations and age groups worldwide. It results to a significant increase in mortality and morbidity related to coronary heart diseases, diabetes type 2, metabolic syndrome, stroke, oxidative stress and cancers [11].

This present study shows that obesity significantly increases fasting plasma glucose, insulin, MDA, blood pressure and it causes insulin resistance ($P < 0.05$). In comparing class II with Class I obese, there were significant increases in FPG, Insulin, HOMA-IR, Systolic and blood pressure ($P<0.05$) except MDA which did not differ significantly ($P>0.05$). This finding is in line with Gurung *et al*, [12] which also showed significant increased levels of fasting serum insulin, insulin resistance in obese group when compared with their controls. This indicates that class II obese groups are at the highest risk of developing atherosclerosis, hypertension and diabetes mellitus. This increase might be as result of link between obesity and impaired serum glycemic levels as a result of different cellular mechanisms including alterations of insulin signalling, changes in glucose transport, pancreatic β cell dysfunction, as well as enhanced oxidative stress (OS) and inflammation [13]. Obese individuals have demonstrated markers indicative of oxidative stress, including elevated measures of reactive oxygen species (ROS) [14] and diminished antioxidant defense, which is associated with lower antioxidant enzymes as a result of increased free fatty acid which inhibits NADPH oxidase causing dysregulation of cytokines leading to insulin resistance. Oxidative stress is associated with systemic inflammation, endothelial cell proliferation and apoptosis, and increased vasoconstriction, and thus a noteworthy contributing factor to endothelial dysfunction. [15]

In this study, significant weight loss was observed only after 12 weeks green tea supplementation when compared with baseline and also in 12weeks when compared with 6weeks supplementation($P<0.05$) unlike waist circumference which reduced significantly after 6 and 12wks intervention when compared with baseline ($P <0.05$). This is in line with an intervention study by Suzuk *et al.* [16] which revealed that subjects with high catechin intake had lower body weight, BMI, abdominal circumference, total abdominal fat area, after 12 weeks than those of the placebo group. In contrast, supplementation with 300 mg/d of EGCG for 12 weeks according to Mielgo-Ayuso *et al,* [17] did not improve weight-loss . However, few others demonstrated that green tea has no effect on FPG [18], there was no glucose or insulin-lowering effects after consumption of 300 mL of green tea or water [19]. Weight reduction by green tea observed in this study might be due to reduced digestibility and an increase in energy expenditure and fat oxidation through β-adrenoceptor activated thermogenesis of brown adipose tissue [20] and also due to inhibition of catechol-O-methyl

transferase (COMT) enzyme by epigallocatechingallate (EGCG) of the green tea [21]. Furthermore on 12weeks supplementation in this work, there were significant decrease in systolic blood pressure, fasting plasma glucose, fasting blood insulin, MDA, homeostatic model assessment - Insulin resistance (HOMA-IR) ($P < 0.05$) when compared with their baseline, however FPG, HOMA-IR, MDA and systolic pressure did not significantly decrease after the first 6weeks intervention.($P > 0.05$) Furthermore diastolic blood pressure did not reduce significantly throughout the 12weeks supplementation($P > 0.05$). This finding is in line with work done by other researchers [22] [23]. This is also in line with the work of Liu *et al*. [24] which also showed that green tea extract caused a significant decrease in homeostasis model assessment of insulin resistance index after 16 weeks. The anti-hyperglycemic effect of green tea as seen in this study might be as a result of the increase in insulin-stimulated glucose uptake, inhibition of the intestinal GLUT system and decrease in expression of genes that control gluconeogenesis . Mozaffari-Khosravi *et al.* found out significant decrease in systolic and diastolic blood pressure on individuals who consumed three glasses of green tea daily for 4 weeks. However, in this present study green tea did not reduce blood pressure at 6weeks supplementation. This might be as a result of GT dosage or rate of consumption, also the brand of tea used should be considered. The decreased blood pressure found in this study by green tea is because it regulates vascular homeostasis by its influence on the production of angiotensin II, prostaglandins, endothelin-1 as well as vasodilating substances such as prostacyclin [25]

Conclusion

In conclusion, increase in severity of obesity as seen in class II obese individual exposes the individual to more complications and health related problems of obesity especially hypertension, oxidative stress and diabetes mellitus and subsequent supplementation with green tea for 12weeks can ameriorate these disorders caused by obesity.

Consent

All authors declare that ‘written informed consent was obtained from the subjects and other approved parties for publication of this paper and accompanying images.

Ethical approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee (the ethical review committee of the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki ”ethical

REFERENCES

1. Adedayo OS, Ebenezer OO. Prevalence of obesity among adolescents in Ile-Ife, Osun state, Nigeria using body mass index and waist hip ratio: A comparative study. 2013; 54(3) 153-156.
2. Akiibinu MO, Soile BO, Ajibola M, Amzat U, Olatunji TK. Plasma Levels of CA125, CEA, AFP and Cortisol in Obesity. *Journal of Steroids & Hormonal Science*. 2015; 4:2.
3. Rossner S. Obesity: the disease of the twenty-first century. International Journal of Obesity Related Metabolic Disorder. 2002; 26(Suppl 4):S2–4.
4. Mann J, Truswell S. Essentials of human nutrition. Oxford Univ. Press. 2012; 69:316.
5. *Wu AH, Spicer D, Stanczyk F.Z, Tseng C.C, Yang C.S, Pike M.C; Effect of 2-month controlled green tea intervention on lipoprotein cholesterol, glucose, and hormone levels in healthy postmenopausal women. Cancer Prev Respondent. 2012; 5:393–402*
6. Kelly Da, Nelson R. Characterization and quantification of Phytochemicals by gas chromatography. *Journal of Brazil*. Chemistry. Society. 2014; Vol 25.
7. Lewis SM, Bain B.J, Bates I, Dacie JV. Dacie and Lewis practical Haematology, Philaldephia Churchill Living stone. 2006
8. Trinder P; Determination of blood glucose using an oxidase- peroxidase system with a non carcinogenic chromogen. *Journal of Clinical Pathology.*1969.
9. Gutteridge J.M and Wilkins S. Copper dependant hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. 1982; FEBS Letts. 137:327-330.

10. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC; Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985; 28: 412–419.

11. Eckel RH, York DA, Rössner S, Hubbard V, Caterson I, St Jeor ST. American Heart Association. Prevention conference VII obesity, a worldwide epidemic related to heart disease and stroke: executive summary. *Circulation*. 2004; 110:2968–2975.

12. Gurung A, Meera S, Ebenezer W. Study of insulin resistance and lipid profile in obese adults. *International Journal of Science Innovations and Discoverie*. 2013; 3 (3) , 3 6 2 - 3 6 6 .

13. Fernández-Real JM, Broch M, Vendrell J, Ricart, W. Insulin resistance, inflammation, and serum fatty acid composition," *Diabetes Care*. 2003; vol. 26, no. 5, pp. 1362–1368.

14. Keaney JF, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Obesity and systemic oxidative stress clinical correlates of oxidative stress in the Framingham Study. *Arteriosclerosis Thrombosis Vascular Biology*. 2003; 23:434–9

15. Huang C, Matthew J, McAllister, Aaron L, Slusher, Heather E. Webb. Obesity-Related Oxidative Stress: the Impact of Physical Activity and Diet Manipulation .*Sports Medicine Open*. 2015; 1: 32.

16. Suzuki Y, Miyoshi N, Isemura M. Health-promoting effects of green tea. *Proc Jpn Acad Ser B Phys Biol Sci*. 2012; 88(3):88–101.

17. Mielgo-Ayuso J, Barrenechea L, Alcorta P, Larrarte E, Margareto J, Labayen I. Effects of dietary supplementation with epigallocatechin-3-gallate on weight loss, energy homeostasis, cardiometabolic risk factors and liver function in obese women: randomised, double-blind, placebo-controlled clinical trial. *British Journal and Nutrition*. 2014; 111(7):1263–1271.

18. Mozaffari-Khosravi H, Ahadi Z, Barzegar K. The effect of green tea and sour tea on blood pressure of patients with type 2 diabetes: a randomized clinical trial. *Journal of Diet Supplement* 2013; 10(2):105–115.

19. Josic J, Olsson AT, Wickeberg J, Lindstedt S, Hlebowicz . Does green tea affect postprandial glucose, insulin and satiety in healthy subjects: a randomized controlled trial? *Nutrition Journal*. 2010; 9:63.

20. Hasani-Ranjbar S, Zahra JH, Mohammad AI. A systematic review of anti-obesity medicinal plants - an update. *Journal of Diabetes and Metabolic Disorder*. 2013; 12: 28.

21. *Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R. Novel insights of dietary polyphenols and obesity. Journal of Nutrition and Biochemistry. 2014; 25(1):1–18.*

22. Peng X. Effect of green tea consumption on blood pressure: a meta- analysis of 13 randomized controlled trials. Science Report 1. 2014; (4): 6251

23. Szulińska M, Marta Stępień, Matylda Kręgielska-Narożna, Joanna Suliburska. Effects of green tea supplementation on inflammation markers, antioxidant status and blood pressure in NaCl-induced hypertensive rat model. Journal of Food & Nutrition Research 2017; Volume 61, - Issue 1.

24. Liu CY, Huang CJ, Huang LH, Chen IJ, Chiu JP, Hsu CH. Effects of green tea extract on insulin resistance and glucagon-like peptide 1 in patients with type 2 diabetes and lipid abnormalities: a randomized, double-blinded, and placebo-controlled trial. 2014; *PLoS One*. 9(3):e91163.

25. Bhardwaj P, Khanna D. *Green tea catechins: defensive role in cardiovascular disorders. Chineese Journal of Natural Medicine.*2013; *11, 345–353*