

Development and Evaluation of an Infusion of Red Tea (*Camellia sinensis*) with Blackberry (*Rubus ulmifolius*) Enriched with β -Glucans for the Control Glycemia in Diabetic Persons

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Abstract

Introduction: An infusion of red tea with blackberries enriched with β -glucans for glycemic control in diabetic subjects was developed and evaluated by virtue of a high percentage of people using plant extracts as traditional medicine to meet their primary health care needs.

Materials and Methods: A completely randomized block design was used to evaluate concentrations of red tea with dehydrated blackberry (50/50 and 75/25), temperatures (75 and 95°C) and infusion times (2 and 5 minutes) for preparation of beverage with higher content of total polyphenols. The selected treatment was evaluated in 30 diabetics for 28 days, who underwent anthropometric measures (weight, height, waist-hip circumference and blood pressure), biometric (glucose and cholesterol) and performed and food intake was analyzed through a 24-hour reminder.

Results: The treatment with higher content of polyphenols was obtained with 75/25 red tea with blackberry, 95°C and infusion for 5 minutes. Tea intake was associated with reductions in anthropometric measures of Body Mass Index (BMI) and Waist-to-Hip Index (WHI).

Conclusion: It was possible to extract greater content of total polyphenols with longer infusion temperature and time. There was a greater reduction in BMI at ages 45 - 65 years, glucose levels at ages 45 - 65 and > 65 years, total cholesterol levels at ages 45 - 65 years and diabetic patients had a normal distribution of dietary intake macronutrients.

Keywords: Blood Cholesterol; Waist-Hip Index (WHI); Body Mass Index (BMI); Total Polyphenols; Blood Pressure; Gallic Acid

Abbreviations

BMI: Body Mass Index; WHI: Waist-to-Hip Index; WHO: World Health Organization; DM: Diabetes Mellitus; DMT1: Type 1 Diabetes Mellitus; DMT2: Type 2 Diabetes Mellitus; CRD: Completely Random Design

Introduction

Honduras has a current population of 8,143,564 inhabitants and shows a population growth of 2.1% [1]. It is characterized as a country of medium and low economic income where 66.2% of Hondurans live in poverty (21.0% in relative poverty and 45.20%

in extreme poverty) the average income is below \$ 200 per month [2], recently there has been an increase in chronic non-communicable diseases, among which they reward obesity and diabetes in the population [3].

The World Health Organization (WHO) establish that across the world, a high percentage of countries use traditional medicine as the mainstay of health care and the major part of the remedy involved plant extracts or their active compounds [4].

Plant-based foods are composed of phenolic, terpenic and sulfur substances, where phenolic compounds are responsible among other things for the organoleptic properties of food. Within these compounds, anthocyanins are responsible for red, blue and violet tones that are characteristic in fruits such as the blackberry [5,6]. Polyphenols, especially phenolic acids and flavonoids, have been considered as active components in the prevention of cardiovascular diseases, cancers, neurodegenerative diseases, or diabetes due to their antioxidant, anti-inflammatory and antineoplastic capacity therefore an increase in their consumption could reflect active benefits [7,8].

Infusions made from the tea plant (*Camellia sinensis*) are the second most consumed beverage in the world after water, with a sales increase of 16% in recent years [9]. Red tea (*Camellia sinensis*) contains a large amount of natural polyphenols including catechin, epicatechin, quercetin and gallic acid among others [10,11]. The blackberry (*Rubus ulmifolius*) native to Central America, spreading throughout the American tropics and the rest of the world, with approximately 400 species; It is also rich in minerals, vitamins and antioxidants in addition has a characteristic smell, slight sweet taste which increases the acceptability of the fruit in addition to conferring benefits on weight control [12,13].

β -glucans are polysaccharides that are found naturally in the cell wall of various living organisms such as bacteria, fungi, yeasts and plants such as barley and oats [14]. Studies carried out in humans have shown that its consumption has an effect on the reduction of glycemia, insulin levels, cholesterol and the modulation of the immune system [15].

The prevalence throughout the world to diabetes mellitus (DM) has grown from 4.7% in 1980 to 8.5% in 2014 in the adult popula-

tion [16]. Provoking the death of more than 1.5 million people in 2012 and 2.2 million more, because of increasing cardiovascular disease, resulting from insulin deficiency or as a result of the ineffective use of insulin, mostly in people who are obese [17,18].

Honduras has recently registered an increase in chronic non-communicable diseases, among which they reward obesity and diabetes [3]. An estimated 51% of women aged 15 - 49 suffer from overweight and obesity nationwide [19-21]. The prevalence of diabetes mellitus type 2 (DMT2) at the national level in adults over 40 years of age is 7.9% and with altered glucose values of 15.5% [6] a recent study carried out nationwide showed that underlying factors for the prevalence of metabolic syndrome [22].

To the best of our knowledge, no studies have been done on the consumption of this kind of tea infusion in Honduras consequently the present study intends to offer an alternative to this problem, by preparing an infusion with functional characteristics in response to this growing health need. As a result, an experimental design was propose for the preparation of an infusion based on red tea (*Camellia sinensis*) with dehydrated blackberries (*Rubus ulmifolius*) and beta-glucans, in order to analyze its effects on anthropometric values, glucose and cholesterol before and after tea consumption in DMT2 patients.

Materials and Methods

The investigation has been divides in two steps: Elaboration of the infusion and total phenolic content assay and evaluation of the infusion in diabetic persons.

Elaboration of the infusion and total phenolic content assay

Material

Red fermented tea (PU-Erh) was purchased from the Hinrichs Teehus (Netherlands), blackberries were purchased from a local fruit supplier Hortofrutícola (Honduras) and Progal Company (Colombia) donated the β -glucans.

Statistical analysis

The statistical analysis of tea was achieved using completely random design completely random design (CRD) 2 x 2 x 2 factorial arrangement. The factors of interest are percentage of blackberry/red tea (500:50 and 75:25), temperature (75°C and 95°C) and infusion time (2 and 5 minutes). SAS (Statistical Analysis System)

was used to analysis the results of total polyphenols measured. An analysis of variance (ANDEVA) with separation of measurements LMEANS between each treatment ($p < 0.05$) was applied.

Tea preparation

Blackberries dehydration was conducted at 135°C for 24 hours using an Excalibur model 9-tray dehydrator; red tea strands were visually inspected to avoid any foreign materials. Both material were grind using Arthur H. Thomas 32F770 grinder and #6 sieve they were mixed by the given ratio of the mathematical model (P2T2t2), then a 10% of beta-glucans powder was added according to final weight. A total of 2g of tea sample was weighted with a ± 0.02 g precision and sealed in polyethylene plastic bags.

Total phenolic assay

The total polyphenol content was determined in the infusions prepared for each of the red and blackberry tea treatments using the Folin-Ciocalteu method. A calibration curve was made with gallic acid; the values were reported as gallic acid equivalents (AGE mg/L) [23].

Evaluation of the infusion in diabetic persons

Preparation

Before anthropometric and biochemical measurements, the researchers have followed a online course "Human subject Research - IRB - Behavioral Educational Core" from Collaborative Institutional Training (CITI). And the protocol of investigation has been sent and approved by Biomedical Research Ethics Committee (CEIB), (IRB 00003070) of The National Autonomous University of Honduras (UNAH). With the purpose of standardize and reduce errors during anthropometric measurements, the researchers have followed a course from the director or the project, who is instructor of Isak level 1 anthropometry. The anthropometric measurements, biochemical analysis, and the collection of 24h-reminders have been achieved in the Laboratory of Human Nutrition of Zamorano University (LNHZ).

Experimental design of the study

A phase 3 intervention and observational clinical trials was carried out in the village of el Jicarito, Department of Francisco Morazan, Honduras, to patients between the ages of 18 and 70 years form both sex diagnosed with DMT2. The data were collected in September 2017; the research has been funded by Institute for Technology in Health Care (ITHC).

Study population

The study was carried out in the village of El Jicarito, Department Francisco Morazán, Honduras. The sampling was not probabilistic with the desire to participate and signed the informed consent. The selection of the participants was those diagnosed with diabetes with referral from the clinic service of the El Jicarito Local Health Center with assistance to the diabetic club. The center reported an estimated 70 new cases of DMT2 for 2017, participants were recruited during the month of March to May 2017.

$$n = \frac{z^2 p \cdot q \cdot N}{Ne^2 + Z^2 p \cdot q} \quad (1)$$

The formula gave the sample size of 34 persons with 95% confidence and 5% of error. Where:

Z: Confidence Level

p: Population proportion

q: 1-p

N: Population size

e: Margin error

n: Sample size.

Inclusion criteria

Patient diagnosed with DMT2 from referral from the general clinic service of the health center, who read and accept the informed consent, between 18-70 years of age, and who have the ability to understand.

Exclusion criteria

Patients under 18 years of age diagnosed with Type 1 Diabetes Mellitus (DMT1), diabetic pregnant women, with cognitive impairment. With 4 participants excluded, the total sample was $N = 30$.

Delivery of informed consent and instructions for preparing the infusion

Prior to data collection, each participant was given an informed consent, stating the objectives and procedures of the investigation, emphasizing the risks and benefits of participating, indicating that participation was totally voluntary and that no monetary or other benefits were offered, having the total freedom to withdraw when deemed appropriate. Together, each participants was given the in-

structions for the preparation of the infusion, indicating that the entire contents of the tea package (2g) should be used in a cup of hot water (250 mL), without addition of any sweetener such as honey, panela, sugar or artificial sweetener and to be consumed within 5 minutes in order to achieve the highest intake of polyphenols. It was advised that consumption be fasting, only in the morning hours, for 28 days. During the treatment, at each appointment made to the diabetic club, participants were reminded not to change their diet, to program physical activity, or to stop the prescribed medication treatment, furthermore sport and physical activity questionnaire were not used to assess changes in physical activity, as it is a phase II clinical trial, it was simply investigated if they complied with the recommendations for physical activity for diabetics.

Data collection

Anthropometry

For each participant the anthropometry data of height, weight, waist, hip and blood pressure were obtained. For anthropometric sampling, equipment with scientifically validated mark was used. For the size measurement, a Seca 212 brand stadiometer was used, and for the weight, a Seca 803 brand digital scale was used. With data obtained, the calculation of the Body Mass Index (BMI) were performed using equation 2. According to the ranges stipulated by the World Health Organization (WHO) [7], the nutritional status of the people evaluated was determined [5,6,12] (Table 1).

$$IMC = \frac{Weight (kg)}{Height (m^2)} \quad (2)$$

Category	Range (kg/m ²)
Below Normal Weight	< 18.5
Normal Weight	18.5 - 24.99
Overweight	25 - 29.99
Obesity I	30 - 34.9
Obesity II	35 - 39.9
Extreme Obesity	> 40

Table 1: BMI Classification.

NHLBI (National Heart, Lung and Blood Institute). Identification, Evaluation, and Treatment of Overweight and Obesity in Adults Clinical Practice Guideline. SH. 2012. Disponible en: <https://ct1.medstarhealth.org/content/uploads/sites/43/2014/12/Identification-evaluation-and-treatment-of-Overweight-and-Obesity>.

As a predictor of cardiometabolic risk the hip-waist index (WHI) was taken. For this an inextensible measuring tape was used measuring the waist circumference at the height of the last floating rib, and the maximum hip perimeter at the level of the buttocks. The calculation of the WHI was performed using equation 3.

$$WHI = \frac{Waist (cm)}{Hip (cm)} \quad (3)$$

Risk of disease	Men	Women
Low	< 0.90	< 0.75
Moderate	0.91 - 0.99	0.76 - 0.84
High	> 1	> 0.85

Table 2: Risk of disease classification according to WHI.

OMS (Organización Mundial de la Salud). Waist Circumference and Waist Hip- Ratio. Report of a World and Health Organization Expert Consultation. 2011; 46p.

Based on the WHI ranges stipulated by WHO [13], the risk of cardiometabolic disease of the participants was determined [24].

The blood pressure measurement was performed with the OMRON electric blood pressure monitor. According to the ranges stipulated by the WHO, the person's pressure range was determined [5] (Table 3).

Range	Systolica (mm/Hg)	Diastolic (mm/Hg)
Optimum	< 120	< 80
Normal	< 130	<85
Stage 1 Hypertension	131 - 159	86 - 99
Stage 2 Hypertension	160 - 179	100 - 109
Stage 3 Hypertension	>180	> 110
Isolated systolic hypertension	>140	< 90
Supine Hypertention	>140	> 90

Table 3: Blood pressure classification.

NHLBI The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. JAMA 2003;289:2560-71.

Biochemical measurement

The biochemical measurements were taken with at least 8 hours fast requirement and without smoking. Participants were asked to attend health center for reasons of displacement and location of the health center; it was not possible to take plasma data. Therefore for the measurement of blood glucose the Accu-check rapid test portable device was used, likewise for the measurement of total cholesterol the Accutrend rapid test portable device was used, in both cases the values obtained were compared according to the ranges stipulated by the WHO [25,26].

Food anamnesis

With the objective of being able to evaluate the dietary intake, a food intake reminder was made, the same stipulated on the first and last Wednesday during the data collection. The format established was completed by means of a directed questionnaire composed of questions asking the participant to remember the intake of the previous day as a usual day; no surveys were taken on Monday or after regional holidays so that there was no variability in the data. They were processed with the program “The Food Processor” SQL version 10.10 for subsequent analysis of macronutrients (Carbohydrates (CH), Proteins (Pr), Fatty Acid (FA)) and micronutrients (Folates, Fe, Ca, Mg, Zn, Vit A, B12, and C).

Analysis of the anthropometric and biochemical analysis blood pressure measurement

A Completely Random Design (CRD) was used with repeated measurements in time per day 0 and 28 after tea consumption. The variables BMI, WHI, Blood Pressure (BP), Blood Glucose and Total Cholesterol were analyzed with the Statistical Analysis System (SAS®) program. A t-test was performed for paired samples; the data were obtained through two measurements made in the same experimental unit (start and end).

Results

Analysis of total polyphenols

The highest concentration of polyphenols was obtained by the P2T2t2 treatment (75% tea, 25% blackberry, 95°C infusion and 5 minutes) (Table 4).

Sociodemographic aspects

The population was comprised of 83% of the female sex and 17% of the male sex. The average age of the total population was

Treatment	Polyphenols Value (mgAG/l) (Mean $\pm$ SD [©])
P ₁ T ₁ t ₁	219.89 $\pm$ 3.83 ^b
P ₁ T ₁ t ₂	218.33 $\pm$ 8.33 ^b
P ₁ T ₂ t ₁	193.36 $\pm$ 10.23 ^{cd}
P ₁ T ₂ t ₂	183.10 $\pm$ 2.81 ^d
P ₂ T ₁ t ₁	238.00 $\pm$ 7.51 ^a
P ₂ T ₁ t ₂	207.78 $\pm$ 4.91 ^{bc}
P ₂ T ₂ t ₁	168.05 $\pm$ 26.50 ^e
P ₂ T ₂ t ₂	241.11 $\pm$ 11.00 ^a
CV [©] (%)	4.03

Table 4: Analysis of total polyphenols of red tea treatments with blackberry.

^{a-e}: Means followed with the different letter are statistically different (P < 0.05).

©: Standard deviation.

[©]: Coefficient of variation.

P₁: 50% of red tea and 50% of blackberry.

P₂: 75% of red tea and 25% of blackberry.

T₁: 75°C temperature for infusion.

T₂: 95°C temperature for infusion.

t₁: 2 minutes of infusion

t₂: 5 minutes of infusion.

54 years with a minimum of 21 years and a maximum of 75 (SD 16.4), 27.5% of the population is less than 45 years old, 40% is less than 45 - 65 years old, and 32.5% is above 66 years old, 66% reported using metformin with an average dose of 1450 mg/ds (SD 350), 6% reported using only insulin and 28% doses of metformin (Table 5).

Body mass index (BMI)

The initial mean population weight was 74.2 kg, the average weight for the female sex was 74.2 kg and 89.6 kg for the male sex. The average population size was 1.56 m, the average for the female sex was 1.56m and for the male sex it was 1.58 m (Table 2). After 28 days of treatment, the average weight for women was 73.8 kg, the male sex reported an average of 89.7 kg.

At the population level, BMI was placed in grade I obesity with a value of 30.4, 37.7% in overweight, 10% in obesity I, 26.7% in

	Initial	Final
Variables	Mean $\pm$ SD	Mean $\pm$ SD
Age (year)	54.00 $\pm$ 16.40	54.00 $\pm$ 16.40
Height (m)	1.56 $\pm$ 0.10	1.56 $\pm$ 0.10
Weight(kg)	74.22 $\pm$ 17.10	73.80 $\pm$ 17.03
BMI	30.35 $\pm$ 5.64	30.23 $\pm$ 5.89
WHI	0.95 $\pm$ 0.06	0.92 $\pm$ 0.07
DBP (mmHg)	141.57 $\pm$ 19.66	143.83 $\pm$ 21.81
SBP (mmHg)	81.33 $\pm$ 11.92	82.60 $\pm$ 12.27
Glucose (mg/dl)	199.23 $\pm$ 104.36	210.00 $\pm$ 117.97
Cholesterol (mg/dl)	201.40 $\pm$ 41.74	206.50 $\pm$ 32.94
Metformin (mg)	1335.71 $\pm$ 587.19	1335.71 $\pm$ 587.19
Insulin (UI)	27.50 $\pm$ 15.00	27.50 $\pm$ 15.00

Table 5: Results of initial anthropometric and biochemical variables.

obesity II and 6.7% in morbid obesity, only 20% found a BMI within the normal range [24]. In the distribution by sex, 36% of the female population reported an overweight BMI, while 80% of the male population was overweight and obese. Post consumption it was observed that the population BMI was 30.3. However, there were changes in normal BMI from 20% to 26.7%, and overweight was 23.3%, although these findings were not statistically significant (Figure 1).

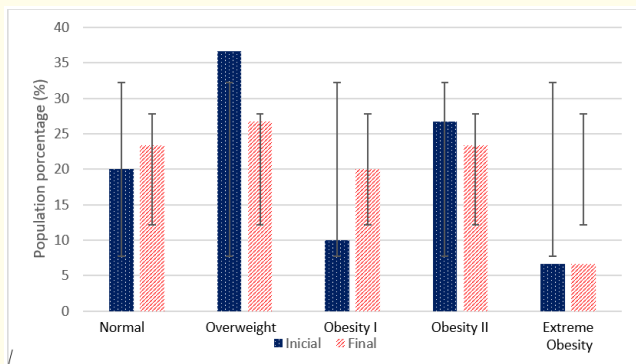


Figure 1: Nutritional status according to the Body Mass Index on the first day and after 28 days of intake of red tea with blackberry.

Waist-hip index (WHI)

At the beginning of the treatment, the average WHI was 0.95 (Table 2), for the male sex it was 0.94 (Figure 2a) and 0.95 for the female sex (Figure 2b) after tea consumption only differences were observed, although no statistically significant ($p = 0.0513$).

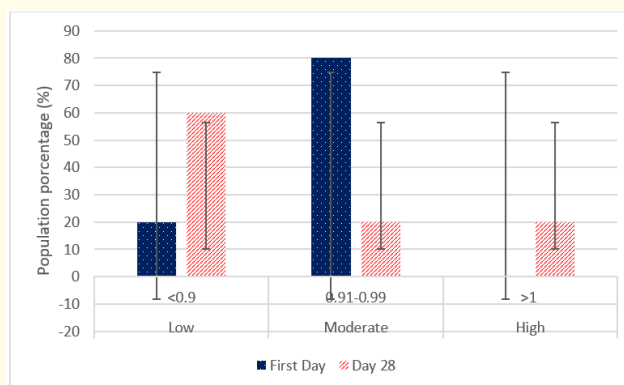


Figure 2a: Risk of cardiometabolic disease in diabetics according to the Waist-Hip Index (WHI) on the first day and after 28 days of intake of red tea with blackberry, male sex population.

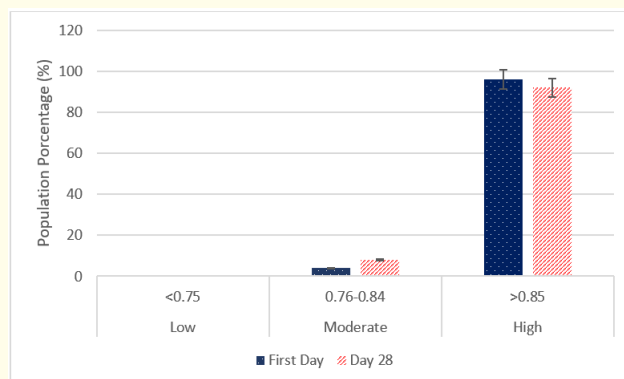


Figure 2b: Risk cardiometabolic disease in the diabetic female population according to the Waist-Hip Index (WHI) on the first day and after 28 days of intake of red tea with blackberry and β -glucan.

Blood pressure measurement

At the beginning of the treatment, the population recorded a mean diastolic blood pressure (DBP) of 141.57 mmHg. (SD 19.66), the record for systolic blood pressure (SBP) was 81.33 mmHg. (SD 11.92), although variations in post-consumption values could be observed, these were not statistically significant (Figure 3).

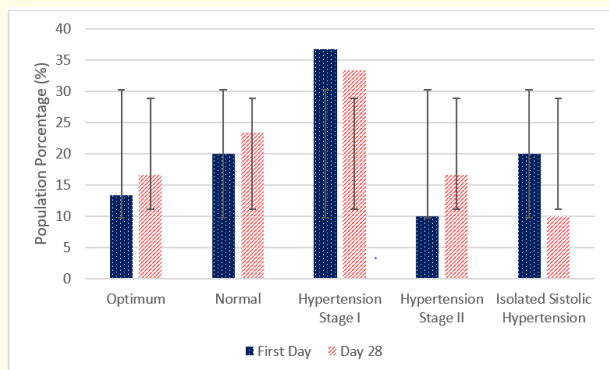


Figure 3: Classification of diastolic and systolic blood pressure in the population of diabetics on the first and after 28 days of intake of red tea with blackberry and β -glucan.

Biochemical measurement

Blood sugar level

At the population level, the average glucose value was 199.3 mg / dl with a (SD = 104.31) post-consumption of tea, statistically significant differences could be observed ($p = 0.0094$), ($p < 0.05$) (Figure 4).

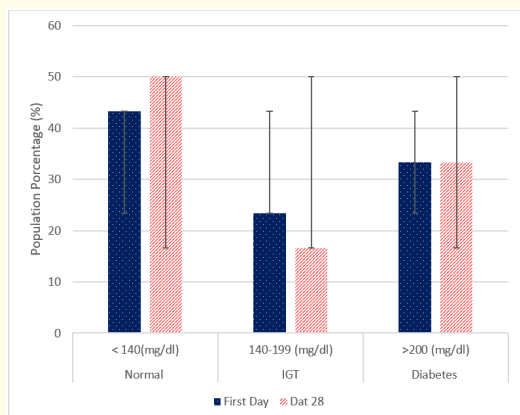


Figure 4: Classification of glucose values in the diabetic population on the first day and after 8 days of intake of red tea with blackberry and β -glucan.

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

Food anamnesis

The average calorie was 1062 of 122.28g for carbohydrates (CH), 45.14g of protein (Pr) and a 42.75g total fat (TF), 11, 03g of dietary fiber and a mean of 798.15 mg of sodium. Consequence of the low socioeconomic status of the region and cultural habits of the population, food selection was represented by basic grains and legumes as protein sources. The consumption of proteins of high biological value derived from meat eggs and dairy products in general, was limited by the high price and low purchasing power of the participants.

From the group of micronutrients, due to their physiological importance, the minerals evaluated were folates, iron (Fe), calcium (Ca), magnesium (Mg), sodium (Na) and zinc (Zn). Vitamins were chosen to evaluate the vitamin (vit) A, B12 and C.

Regarding micronutrients, the average intake at the beginning of the study of folates 114.18 mg, iron 6.94 mg, calcium 320.86 mg, magnesium 373.41 mg; sodium 798.15 mg, zinc 5.17 mg, vitamin A 286.3 mg, B12 1.23 mg and C 36.38 mg, respectively (Table 6).

Total cholesterol

At the population level, the average cholesterol level was 201.4 mg/dl considered an optimal value, no statistically significant differences were observed after consumption (Figure 5) [26].

Discussion

Total phenolic content (TPC)

Gallic acid is a phenolic compound characteristic of red tea and the most important for its notorious bioavailability [27]. The average content of gallic acid in red tea is approximately 151 mg/L and varies depending on the type of tea fermentation, variety and process, time and temperature of sun exposure of the crops, among others [2]. In our study, our results have shown higher polyphenols values, concentrations are comprised between 168 and 241 mg/L of gallic acid. Furthermore, we have concluded that higher temperature (95°C) and higher time (5 minutes) allowed better extraction of polyphenols, this result is consistent with the literature, it has been shown that time, temperature have an effect on the extraction

	Initial	Final
Variables	Mean $\pm$ SD	Mean $\pm$ SD
Energy (kcal)	1061.62 $\pm$ 690.34	1124.72 $\pm$ 514.25
CH (g)	122.28 $\pm$ 82.04	108.69 $\pm$ 90.6
Pr (g)	45.14 $\pm$ 25.15	38.95 $\pm$ 22.4
TF (g)	42.75 $\pm$ 44.85	44.74 $\pm$ 42.65
FA Saturated (g)	17.77 $\pm$ 23.59	23.28 $\pm$ 20.82
FA Monoinsaturated (g)	15.10 $\pm$ 15.99	14.3 $\pm$ 13.87
FA Polyinsaturated (g)	5.51 $\pm$ 4.88	4.16 $\pm$ 5.02
Cholesterol (mg)	233.45 $\pm$ 158.10	385.62 $\pm$ 122.14
Dietetic Fiber (g)	11.03 $\pm$ 7.21	10.1 $\pm$ 8.07
Vitamin A UI	283.44 $\pm$ 285.23	301.12 $\pm$ 286.32
Vitamin B12 (ug)	1.67 $\pm$ 2.19	1.69 $\pm$ 2.14
Vitamin C (mg)	36.38 $\pm$ 42.59	33.15 $\pm$ 38.14
Folate (mg)	114.18 $\pm$ 63.46	116.2 $\pm$ 61.4
Calcium (mg)	320.85 $\pm$ 280.15	314.65 $\pm$ 274.16
Iron (mg)	6.94 $\pm$ 3.91	11.62 $\pm$ 3.71
Magnesium (mg)	373.41 $\pm$ 227.05	385.6 $\pm$ 216.61
Sodium (mg)	798.15 $\pm$ 570.42	796.15 $\pm$ 403.85
Zinc (mg)	5.17 $\pm$ 2.97	5.11 $\pm$ 3.08

Table 6: Results of the analyzed variables, 24-h meal reminder at the beginning of the treatment.

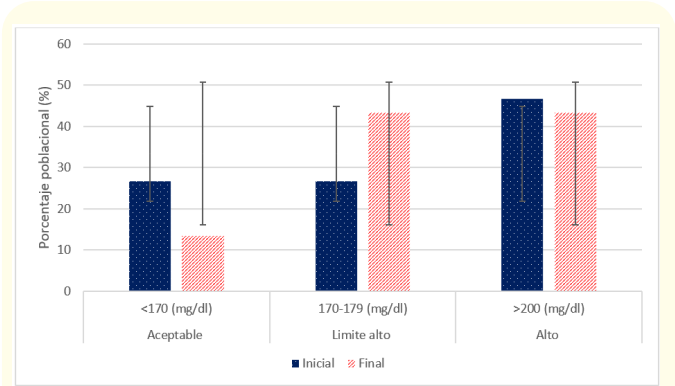


Figure 5: Classification of total cholesterol levels in the diabetic population on the first day and the 28 days of intake of red tea with blackberry and β -glucan.

of tea catechins, and that the majority of epigallocatechin gallate (EGCG) and caffeine are extracted at 85°C for five min [29].

The antioxidants present in red fruits have vasodilator, antipidemic or antisclerotic antithrombotic action. In diabetes, the mechanism of antioxidants exerts action in the small intestine, with gallic acid capable of inhibiting glucose particles, stimulating insulin secretion in the pancreas, activating insulin receptors at the cellular level [30]. Similarly, red tea catechins lower glucose, insulin resistance, reduce chronic systemic inflammation, oxidative stress and improve metabolic cardio risk factors [31-33].

Body mass index (BMI)

BMI reductions are associated with the action of caffeine and catechins contained in red tea, due to their thermogenic properties associated with weight reduction, thus improving body condition [34,35]. It has been shown that the intake of red tea consumption has effects on reducing visceral fat and the accumulation of fat in the liver, accelerating [36,37]. Phenolic compounds and catechins contained in red tea are able to combat free radical action, attenuate cell aging, decrease liver fat and generate a lipolytic effect [17,38,39].

Other studies show that there is an relation between the consumption of blackberries and the reduction of overweight [32,36], this effect is associated with the high content of monounsaturated, polyunsaturated fatty acids, fibers, vitamins and minerals reported by this plant species added to the low content of saturated fats which promotes the prevention of cardiovascular problems, cellular damage and strengthens the immune system [40,41].

The β -glucans are extracted from a fungus called *Ganoderma lucidum* providing excellent benefits on the detoxification of the organism [14,15]. Research confirms finding reduction in BMI values, in a minimum time of dietary or metabolic treatment of three to six months [27-42].

In the present study, changes in the post-consumption BMI were reported, although these were not statistically significant, this variability can be attributed to the short duration of tea intake, to the improvement of glucose levels, hyperglycemia and weight

loss reported; directly affecting body weight and therefore BMI [16,17,43]. Normalizing blood glucose values will therefore result in an increase in weight directly affecting the BMI values (Figure 1).

Waist-hip index in men (WHI)

It is considered a predictor of cardiovascular risk, being widely used in the classification of obesity. As a consequence, variations in the distribution of body mass will depend on sex, age and aging [28].

Physiologically, the males are characterized by a higher proportion of total lean mass, bone mineral mass and a lower proportion of total body fat mass compared to females [44]. Sex differences in body composition are attributable to the action of steroid hormones that drive dimorphisms during pubertal development [32].

Body fat may remain constant or increase with age, but in aging it is associated with a redistribution of fatty tissue between organs [42]. From the middle age to the 80s or more, there is a decline in the volume of subcutaneous fat and a redistribution of fat from subcutaneous to visceral deposits increasing sarcopenia [30-45]. The WHO shows that waist circumference increases with age being higher in the elderly than in young adults until the age of 70 years [31].

A redistribution was observed in the post-consumption WHI parameter, from moderate risk values 0.91 - 0.99 to very low < 0.9, although not statistically significant (Figure 2a) possibly associated with the vasodilator effect and inhibition of LDL oxidation.

Waist-hip index in women (WHI)

In older women there is an increase in androgenicity, measured by the increase in testosterone, causing accumulation of abdominal adipose tissue, increasing hepatic lipase activity and HDL levels [34]. Taken this index also as a simple indicator to evaluate lipid metabolism; in this study it was possible to determine what, as age and aging increases, the waist-hip index values also increase, generating greater morbidity in the development of chronic non-communicable diseases [35,36].

Some authors suggest that polyphenols allow modification of the composition of intestinal microbiota decreasing the absorption of fats [46], forcing the metabolism to mobilize white adipose

tissue, inducing the ability of mitochondria to use stored fat as an energy source avoiding adipogenesis of tissues [47]. Statistically significant differences were observed on post-consumption WHI ($p = 0.034$), with a population redistribution of high risk > 0.85 to moderate 0.76 - 0.84 (Figure 2b).

Blood pressure (BP)

The orthostatic position can generate a drop of 20 mmHg or more in systolic pressure or below 90 mmHg of systolic pressure and a drop of 10 mmHg or more in diastolic pressure or below 60 mmHg. A change in 5 mmHg in either of the two pressures (systolic or diastolic) is associated with an increase in cardiovascular disease by 20 to 30% [39].

However, the present study showed differences in the values of diastolic blood pressure (DBP) and systolic blood pressure (SBP) statistically significant post-consumption, DBP ($p = 0.0009$) and SBP ($p = 0.006$), possibly associated with the benefit of epigallocatechin gallate (EGCG), the main catechin of tea. Research suggests that the intake of this component increases the antioxidant capacity in local vascular tissue, systemic circulation and reduction of atherosclerosis [40-48] (Figure 3).

Blood sugar level (BG)

Catechins metabolize sugar in the body, stimulating the cells of the pancreas to produce and secrete insulin in adequate amounts in order to level blood glucose [49]. Studies show that the consumption of 1.5g of red tea 20 min before oral administration of a dose of glucose significantly reduces blood glucose levels, as a consequence of the inhibitory effect of the catechins present in tea [50]. It was shown that the intake of 300 mg of β -glucans in patients with cardiovascular diseases improved blood circulation, decreased fat content avoiding arteriosclerosis as a result of adenosine and triterpenoids in the ganodic acid [1], together with the movement of glucose and naturally stimulating the metabolism resulting from the synergy of its compounds [27].

In this study glucose values showed significant statistical differences post consumption ($p = 0.001$), the reduction in glucose levels can be associated with the antioxidant properties of tea, improving oxidative stress, insulin pathway, inhibition of digestive enzymes (α -amylase and α -glucosidase), thereby generating a decrease in endothelial dysfunction and inflammation modulation [51] (Figure 4).

Total cholesterol blood level

β -glucans have properties on cholesterol reduction, reducing LDL, due to the presence of triterpenoids and adenosine [52]. The mechanisms of β -glucans incorporated in the regulation of cholesterol homeostasis have the following effects: increased conversion of cholesterol into bile acids, increased cholesterol synthesis and decreased blood cholesterol [25].

The effect that red tea has on reducing cholesterol levels can be associated with EGCGs in the lipid profile of humans [27]. Gallic acid present in red tea leaves and the high content of catechins increases the resistance of plasma LDL [28]. Similarly, the teaflavins contained in red tea are associated with the decrease in the incidence of cardiovascular accidents, atherosclerosis and hypertension, helping to reduce the levels of total cholesterol in the blood and its oxidation [28]. It was observed a post-consumption statistically significant differences ($p = 0.0002$) in total cholesterol blood level, possibly associated with the decrease of the micellar solubility of cholesterol at the intestinal level and therefore reducing its absorption, among other effects already mentioned (Figure 5).

Food anamnesis

Consumption of food with low glycemic indexes has been associated with metabolic control in patients already diagnosed and prevention in the development of DM2 as well, consumption of small amounts of fruits was shown to reduce post-pandemic glucose concentrations, increasing glycogen synthesis hepatic [45].

It is recommended that the distribution of macronutrients on a 2000 calorie basis represented by 55 to 60% of calories ingested by carbohydrates, 13 - 15% by proteins where 50% of them are of high biological value, preferring animal protein with a fat consumption not exceeding 30% of the total calories ingested. [30,54].

According to the Institute of Nutrition of Central America and Panama (INCAP), the food used for human consumption represented by more than 90% of the population in both rural and urban areas and at different levels of poverty are: eggs, rice, beans, sugar and salt; less than 50% of the population reported consumption of beef and in 50% or more of households independent of the level of poverty reported consuming the following products: sugar, eggs, beans, salt, rice, cheeses, poultry, bananas, onions, sauces, tomatoes, citrus fruits, sweet bread, potatoes/roots and vegetable shortening [53].

It could be observed that the population caloric average only covers 50% of the recommended kcal, since the distribution of the macronutrients, the CH accounted for 44%, the Pr 60% and TF 66% of the daily energy requirement (DER). Regarding the CH authors suggest that the consumption should not be less than 150 gr/day in order to avoid ketosis, the average consumption of this macronutrient was 122.27g (71) however, the low consumption of CH has a direct association with dietary and nutritional insecurity associated with economic income.

Regarding micronutrients, calcium intake covered only 32% of the DER, being the most important mineral since bone preservation, nerve transmission and regulation of heart muscle, as well as zinc with 75% of the DER (70). It was possible to observe statistically significant post-consumption differences in the values of CH ($p = 0.0009$), Pr ($p = <.0001$), Fe ($p = 0.0008$), fiber ($p = 0.0006$), Mg ($p = <.0001$) for the rest of the macro and micronutrients no differences were reported (Table 3).

Conclusion

It was concluded that, at a longer time and infusion temperature, there is greater extraction of total polyphenols. Tea intake for 28 days was associated with reductions in anthropometric measures on WHI ($p = 0.034$) only for females, both sexes reported a decrease in the values of DBP ($p = 0.0009$) and SBP ($p = 0.006$). On the biochemical values, the intake of the infusion showed to have inference in the values of glucose ($p = 0.001$) and cholesterol ($p = 0.0002$) respectively. Post-consumption dietary intake showed statistically significant differences were observed in the values of CH ($p = 0.0009$), Pr ($p = <.0001$), Fe ($p = 0.0008$), fiber ($p = 0.0006$) and Mg ($p = <.0001$).

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Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analysis or interpretation of data, in writing of the manuscript or in the decision to publish results.

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