The anti-obesity potential of *Ilex paraguariensis*: results from a meta-analysis

Ângelo Filipe Santos Luís[1], Fernanda da Conceição Domingues[1], Luísa Maria Jota Pereira Amaral[2],*

[1]*Centro de Investigação em Ciências da Saúde (CICS-UBI), Universidade da Beira Interior, Covilhã, Portugal,* [2]*Centro de Matemática e Aplicações (CMA-UBI), Universidade da Beira Interior, Covilhã, Portugal*

The dried leaves of *Ilex paraguariensis*, also known as yerba mate or chimarrão, are used to produce infusions consumed by over 1 million people worldwide, being a traditional drink in several South American countries, particularly, Argentina, Brazil, Uruguay, and Paraguay. The positive effects of *I. paraguariensis* in obesity have been described and observed in some clinical trials both in animals and in humans. The main goal of this work was to perform a systematic review, complying with the PRISMA (Preferred Reported Items for Systematic Reviews and Meta-Analysis) recommendations, followed by a meta-analysis and Trial Sequential Analysis (TSA), to summarize the results of the recent clinical trials, clarifying the anti-obesity potential of *I. paraguariensis*. The search for this systematic review was performed on several electronic databases (Pubmed, Web of Science, Scopus, SciELO and Cochrane Library) in July 2017. A total of 22 clinical trials were considered for the quantitative synthesis of the data, totalizing 726 patients enrolled. In conclusion, this systematic review demonstrated the positive effects of *I. paraguariensis* consumption on the reduction of body weight, body mass index (BMI), and waist circumference of patients, showing its anti-obesity potential.

Keywords: *Ilex paraguariensis*/systematic review. Meta-analysis. Clinical trials. Anti-obesity.

INTRODUCTION

The dried leaves of *Ilex paraguariensis*, also known as yerba mate or chimarrão, are used to produce infusions consumed by over 1 million people worldwide, being a traditional drink in several South American countries, particularly, Argentina, Brazil, Uruguay, and Paraguay (Kim *et al*., 2015). More recently, the aqueous preparations of *I. paraguariensis* have also been consumed in North America and Europe (Kim *et al*., 2015). The dried and minced leaves of *I. paraguariensis* are used to make brewed tea, which is often used both as a source of caffeine, but also as a therapeutic agent because of its pharmacological properties (Bracesco *et al*., 2011). The concentration of caffeine varies among the different types of tea (green tea: 130 mg/L; white tea: 140 mg/L; black tea: 89 mg/L) and coffee (70 mg/L), being the

infusion of *I. paraguariensis* a great source of this compound (40 mg/L) (Rostagno *et al*., 2011).

The recent interest in *I. paraguariensis* is linked to its high content of phytochemicals and the peculiarity of its phenolic profile, characterized by the wealth in mono and dicaffeoylquinic acids, known for their biological activities (Cardozo Junior, Morand, 2016). It has also been shown that infusions of *I. paraguariensis* increase the antioxidant protection of plasma and cholesterol particles against *ex vivo* copper-mediated lipid peroxidation in humans, together with the improvement in the plasma lipid profile of normolipidemic and healthy dyslipidemic individuals (Boaventura *et al*., 2012; Morais *et al*., 2009). Furthermore, the consumption of *I. paraguariensis* beverages, seems to be related to an improvement of glycemic control on pre-diabetic subjects (Klein *et al*., 2011). The positive effects of *I. paraguariensis* in obesity have been observed in some clinical trials both in animals and in humans (Pontilho *et al*., 2015; Gambero, Ribeiro, 2015; Kim *et al*., 2012).

Overall, 23.2% of the world's adult population in 2005 was overweight, with 9.8% being considered

*Correspondence: L. Pereira. Centro de Matemática e Aplicações (CMA-UBI), Universidade da Beira Interior. Rua Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal. Tel.: +351 275 319 700; Fax: +351 275 329 183. E-mail: lpereira@ubi.pt

obese (Kim *et al.*, 2012). The estimated total number of overweight and obese adults in 2008 was 1500 million and 500 million, respectively (Kim *et al.*, 2012). Moreover, in 2016, more than 1.9 billion adults, 18 years and older, were overweight, being 650 million of these considered obese (WHO, 2018). Additionally, 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese (WHO, 2018). Since the prevalence of obesity is a major public health concern, due to all the weight-related diseases that result in significant morbidity and mortality, and reduced quality of life, it is important to search for new strategies to help obese people reducing their body weight (Gambero, Ribeiro, 2015). *I. paraguariensis* appears as a potential new natural approach to treat obesity, being observed that *I. paraguariensis* modulates the expression of genes that are changed in the obese state and restores them to normal levels of expression (Gambero, Ribeiro, 2015).

Despite the current research focused on the bioactive properties of *I. paraguariensis*, which has led to an increase in clinical trials in humans, contradictory results were frequently reported. Taking this context into consideration, the main goal of this work was to perform a systematic review, complying with the PRISMA (Preferred Reported Items for Systematic Reviews and Meta-Analysis) recommendations, followed by a meta-analysis and Trial Sequential Analysis (TSA), to summarize the results of the recent clinical trials, clarifying the anti-obesity potential of *I. paraguariensis*.

MATERIAL AND METHODS

Search strategy, inclusion criteria and study selection

The search for this systematic review was performed on several electronic databases (Pubmed, Web of Science, Scopus, SciELO and Cochrane Library) in July 2017. The databases were queried using the Boolean operator tools, with the following strategy: (Ilex paraguariensis OR yerba mate OR yerba maté OR chimarrão) AND (clinical trial OR trial) AND human. The list of references of the articles reviewed were also checked to find additional studies. Moreover, several academic theses of the authors of some articles were also found (Google Scholar) and were used to complement the gathering of the results. Following the PRISMA recommendations (Moher *et al.*, 2015), titles and abstracts of records retrieved were initially screened and the full texts of those considered relevant were then downloaded and analysed in detail. Two authors independently conducted the literature selection process, with a third being consulted in case of discrepancies. To be included in this systematic review, studies must accomplish the following criteria: to be clinical trials in humans, to present a true control group, to show the results of the outcomes at the baseline and at the end of the trial, and to indicate the standard deviation (SD) of the measurements.

Data extraction and synthesis

After the selection of the studies, the clinical trials included were carefully analyzed and the following data were extracted and summarized: first author's last name, year of publication, location, study design, number of patients (intervention and control groups), type of patients, mean age and gender of patients, study duration, dosage of *I. paraguariensis* and the list of outcomes of interest. According to the PRISMA methodology (Moher *et al.*, 2015), two authors independently reviewed and extracted the data using a prespecified protocol. In cases of discordance, a third reviewer was consulted to analyse discrepancies in data extraction.

Risk of bias assessment

The risk of publication bias of each included study was assessed using the "Cochrane Guide for Review Authors on Assessing Study Quality" which is based on "Cochrane Collaboration's tool for assessing risk of bias" (Ryan *et al.*, 2013). This tool classifies the risk of bias in randomized controlled trials (RCTs) included in reviews as "High risk", "Unclear" or "Low risk" in accordance with 7 domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other sources of bias (Higgins *et al.*, 2011). This classification was independently assigned by two authors and discrepancies in assessment were resolved through discussions between the authors or by consultation of a third investigator. The results of the risk of bias assessment are presented in a risk of bias summary and in a risk of bias graph, which were sketched using the software Review Manager 5.3 (Version 5.3.5).

Statistical analyses

For each outcome of interest, an assessment was performed on the pooled effect of the different interventions in terms of weighted mean differences (WMD) between the change from baseline and post-treatment mean values. Data statistical analysis was performed using Comprehensive Meta-Analysis software (Version 2.0) by introducing the number of patients, the differences in mean and respective

SD values of the outcomes for intervention and control groups. Forest plots were generated to illustrate the study-specific effect sizes along with a 95% confidence interval (CI). The random effects model was used, however, when a fixed effects model would be more useful, i.e., when there was little variance in effect sizes, the software automatically converted the random effects model into a fixed effects model (Borenstein, Hedges, Higgins, 2009). The statistic I^2 of Higgins was used as a measure of inconsistency across the findings of the included studies. The scale of I^2 has a range of 0 to 100% and values on the order of 25%, 50% and 75% are considered low, moderate and high heterogeneity, respectively (Higgins *et al.*, 2003). Three different analyses were used to assess the potential impact of publication bias on the present meta-analysis. The first analysis is a funnel plot (Light, Singer, Willett, 1994; Light, Pillemer, 1984). The symmetry of the funnel plots in which mean differences were plotted against their corresponding standard error (SE) was assessed. In the absence of publication bias, the studies were symmetrically distributed in relation to the pooled effect size. Given that the interpretation of a funnel plot is largely subjective, Egger's regression test was performed to test for potential publication bias (Egger *et al.*, 1997). Since this test tends to have lower power, the absence of a significant correlation cannot be taken as evidence of symmetry (Borenstein, Hedges, Higgins, 2009). Therefore, another analysis was used based on the approach of Duval and Tweedie's Trim and Fill (Duval, Tweedie, 2000a; 2000b). Trim and Fill uses an iterative procedure to remove studies that contain a small sample size from the positive (or negative) side of the funnel plot. It then re-computes the effect size at each iteration until the funnel plot is symmetrical in terms of the new effect size, yielding an unbiased estimate of the pooled effect size. This approach allows the best estimate of the unbiased pooled effect size to be obtained and lends itself an intuitive visual display as it creates a funnel plot that includes both the observed studies and the imputed studies.

The sensitivity analysis was also performed by removing each study, one at a time, to evaluate the stability of the results.

Meta-regression analysis was also undertaken to evaluate the association between calculated WMD in body weight, body mass index (BMI) and waist circumference, and the study duration (Borenstein, Hedges, Higgins, 2009).

Trial Sequential Analysis

TSA is a methodology that uses a combination of techniques in which the evidence required is quantified,

providing a value for the required information size (RIS) (Thorlund *et al.*, 2011). The TSA program version 0.9.5.5 Beta (Copenhagen Trial Unit, www.ctu.dk/tsa/downloads/aspx) was used to adjust the confidence intervals due to sparse data and repeated testing in cumulative meta-analysis. If the cumulative *Z*-curve crosses a trial sequential monitoring boundary or enters the futility area, then it can be concluded that a sufficient level of evidence for the anticipated intervention effect may have been reached and no further trials are needed; whereas if the *Z*-curve does not cross any of the boundaries and the RIS has not been reached, there is insufficient evidence to reach a conclusion. The outcome variables in the present meta-analysis were continuous, so the RIS was estimated based on the empirical data auto-generated by the software per the data input. The TSA was performed at the level of an overall 5% risk of type I error and a power of 20%.

RESULTS AND DISCUSSION

Search and selection of studies

The progression details of the electronic search for this systematic review are represented as a flow-diagram (Figure 1). From the 153 eligible articles, 8 were included in this systematic review with meta-analysis. Among the reasons for exclusion of articles were mostly the lack of a true control group and the absence of SD values for the outcomes of interest. The work of Kim *et al.* (2012) was divided into 2 different trials because the authors used two durations for the intervention (3 and 6-weeks). A similar procedure was undertaken in the trial of Kim *et al.* (2015) (6 and 12-weeks). The study of Klein *et al.* (2011) was divided into 6 different trials taking the possible combinations between the study duration (20, 40 and 60-days) and the type of patients enrolled in the trial (type 2 diabetes and pre-diabetes) into account. The work of Morais *et al.* (2009) was divided into 8 different trials, considering the different durations of the study (20, 40, 60 and 90-days) along with the treatment of the dyslipidemic patients with or without statin. As a result, a total of 22 clinical trials were considered for the quantitative synthesis of the data, totalizing 726 patients enrolled.

Included studies and trials characteristics

The main characteristics of the included trials are summarized in the Table I. The studies cover almost a decade, from 2007 to 2016. The included trials were mostly performed in Brazil and in the Republic of Korea. It will be important to evaluate the effects of *I. paraguariensis*
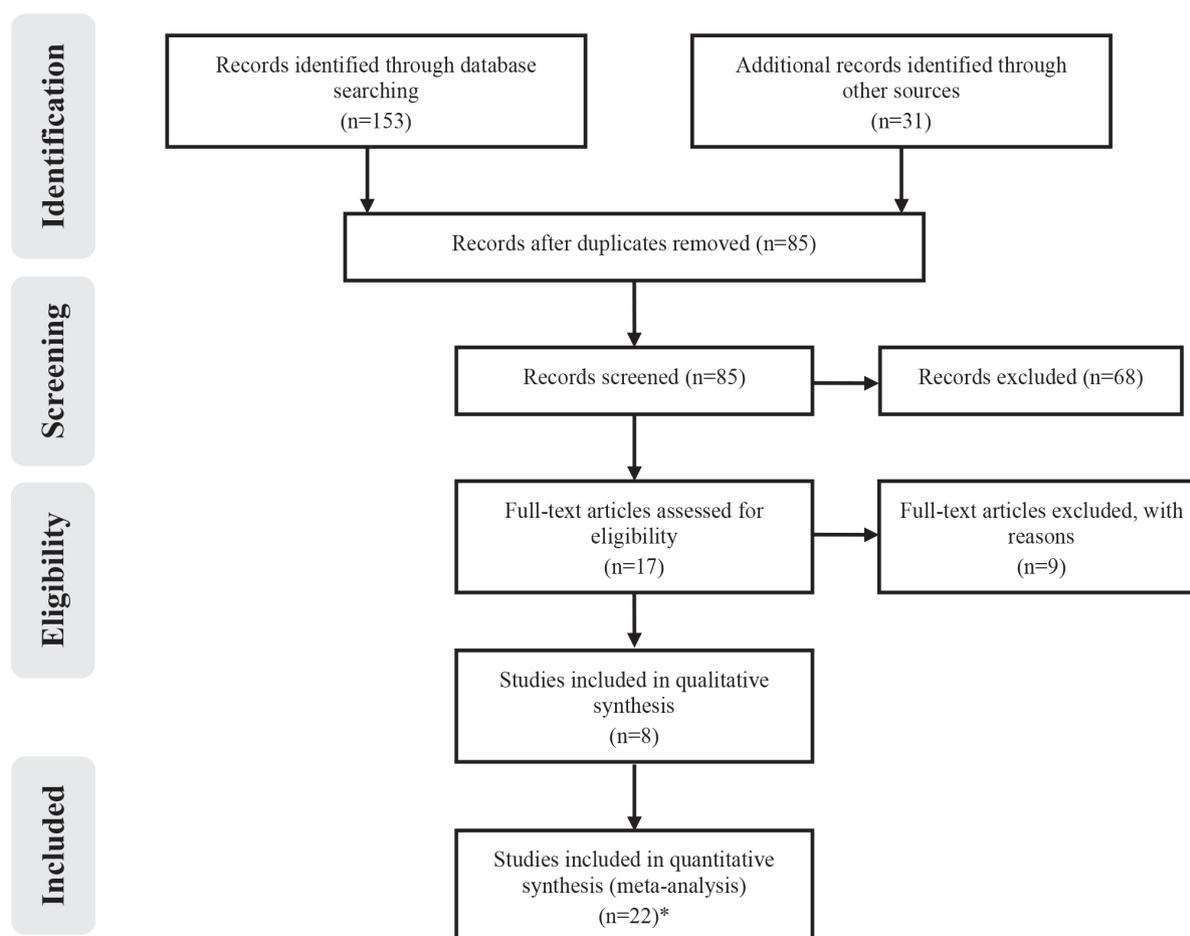
Identification

| Records identified through database searching (n=153) | Additional records identified through other sources (n=31) |

Screening

Records after duplicates removed (n=85)

Records screened (n=85) → Records excluded (n=68)

Eligibility

Full-text articles assessed for eligibility (n=17) → Full-text articles excluded, with reasons (n=9)

Studies included in qualitative synthesis (n=8)

Included

Studies included in quantitative synthesis (meta-analysis) (n=22)*

FIGURE 1 - Flow-diagram of database search, trial selection and articles included in this meta-analysis. *The works of Kim *et al.*, 2012 and Kim *et al.*, 2015 were divided into 2 different trials. The work of Klein *et al.*, 2011 was divided into 6 different trials. The work of Morais *et al.*, 2009 was divided into 8 different trials.

consumption on patients from other countries, as several other factors may interfere in the biological effects when consuming polyphenol-rich plants. For example, the bioavailability of the bioactive compounds, and particularly the polyphenols, is extremely affected by their metabolization, which in turn is influenced by the genotype characteristics of a certain region of the world (Rein *et al.*, 2013). The patients subjected to the intervention with *I. paraguariensis* were mainly dyslipidemic, obese and diabetic. However, it will be necessary to evaluate the effects of *I. paraguariensis* also in healthy volunteers due to the high concentrations of caffeine found in this plant. *I. paraguariensis* was administered to the patients mainly by aqueous preparations, varying the duration of the interventions and the dosage.

Risk of publication bias

The results found in the assessment of the risk of publication bias from the included studies are summarized in Figure 2. The studies of Morais *et al.* (2009) (from A to H) and Salerno (2007) were non-randomized and for that reason were not classified to their risk of bias since the Cochrane's tool can only be applied to RCTs. In general, all the trials satisfied the 7 domains of bias defined by Cochrane Collaboration. The included RCTs claimed to be randomized, but only 6 trials detailed the randomization process and so were classified as "Low risk" in the random sequence generation domain. Concerning the performance and detection bias, related to blinding of participants and personnel, and blinding of outcome assessment, respectively, there were 2 studies (Boaventura *et al.*, (2012), and Pontin (2010)) classified as "High risk", since there were doubts regarding the blinding process (single or double). It is important to note, however, that the assessment of risk of publication bias is a subjective task, even when employing the Cochrane's tool, because it is based on the personal judgments of the review authors.

TABLE I - Characteristics of the 22 included studies in this review with meta-analysis

Study	Location	Study design	Number of patients	Sample size (Intervention group/Control group)	Type of patients	Mean age	Gender (M/F)	Intervention group	Control group	Study duration	Dosage/day	Outcomes of interest
Boaventura et al., 2012	Brazil	randomized, controlled	51	25/26	dyslipidemic	47.0	9/42	mate tea and dietary intervention	dietary intervention	90-days	1 L of mate tea (20 mg/mL of mate infusions prepared with water)	TC, LDL, HDL, TG
Jung and Hur, 2016	Republic of Korea	randomized, double-blind, placebo-controlled	33	17/16	healthy women	43.6	0/33	mate extract tablet	placebo tablet	6-weeks	3000 mg of mate extract	TC, SBP, DBP, body weight, BMI, waist circumference
Kim et al., 2012 **A)**	Republic of Korea	randomized, double-blind, placebo-controlled	46	24/22	women with BMI ≥ 25 kg/m²	27.5	0/46	gelatin capsules containing green mate powder extract	gelatin capsules containing placebo	3-weeks	3000 mg of green mate extract	TC, HDL, TG, body weight, BMI, waist circumference
Kim et al., 2012 **B)**	Republic of Korea	randomized, double-blind, placebo-controlled	46	24/22	women with BMI ≥ 25 kg/m²	27.5	0/46	gelatin capsules containing green mate powder extract	gelatin capsules containing placebo	6-weeks	3000 mg of green mate extract	TC, HDL, TG, body weight, BMI, waist circumference
Kim et al., 2015 **A)**	Republic of Korea	randomized, double-blind, placebo-controlled	30	15/15	obese	43.2	4/26	Yerba Mate standardized capsules	placebo capsules	6-weeks	1000 mg of yerba mate	TC, LDL, HDL, TG, body weight, waist circumference
Kim et al., 2015 **B)**	Republic of Korea	randomized, double-blind, placebo-controlled	30	15/15	obese	43.2	4/26	Yerba Mate standardized capsules	placebo capsules	12-weeks	1000 mg of yerba mate	TC, LDL, HDL, TG, body weight, waist circumference
Klein et al., 2011 **A)**	Brazil	pilot, randomized, single-blind, controlled	18	9/9	type 2 diabetes	54.7	6/12	mate tea and dietary intervention	dietary intervention	20-days	1 L of mate tea (20 mg/mL of mate infusions prepared with water)	TC, LDL, HDL, TG, SBP, DBP, body weight, BMI, waist circumference
Klein et al., 2011 **B)**	Brazil	pilot, randomized, single-blind, controlled	18	9/9	type 2 diabetes	54.7	6/12	mate tea and dietary intervention	dietary intervention	40-days	1 L of mate tea (20 mg/mL of mate infusions prepared with water)	TC, LDL, HDL, TG, SBP, DBP, body weight, BMI, waist circumference
Klein et al., 2011 **C)**	Brazil	pilot, randomized, single-blind, controlled	18	9/9	type 2 diabetes	54.7	6/12	mate tea and dietary intervention	dietary intervention	60-days	1 L of mate tea (20 mg/mL of mate infusions prepared with water)	TC, LDL, HDL, TG, SBP, DBP, body weight, BMI, waist circumference
Klein et al., 2011 **D)**	Brazil	pilot, randomized, single-blind, controlled	18	10/8	pre-diabetes	59.2	4/14	mate tea and dietary intervention	dietary intervention	20-days	1 L of mate tea (20 mg/mL of mate infusions prepared with water)	TC, LDL, HDL, TG, SBP, DBP, body weight, BMI, waist circumference

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TABLE I - Characteristics of the 22 included studies in this review with meta-analysis (cont.)

Study	Location	Study design	Number of patients	Sample size (Intervention group/Control group)	Type of patients	Mean age	Gender (M/F)	Intervention group	Control group	Study duration	Dosage/day	Outcomes of interest
Klein et al., 2011 **E**	Brazil	pilot, randomized, single-blind, controlled	18	10/8	pre-diabetes	59.2	4/14	mate tea and dietary intervention	dietary intervention	40-days	1 L of mate tea (20 mg/mL of mate infusions prepared with water)	TC, LDL, HDL, TG, SBP, DBP, body weight, BMI, waist circumference
Klein et al., 2011 **F**	Brazil	pilot, randomized, single-blind, controlled	18	10/8	pre-diabetes	59.2	4/14	mate tea and dietary intervention	dietary intervention	60-days	1 L of mate tea (20 mg/mL of mate infusions prepared with water)	TC, LDL, HDL, TG, SBP, DBP, body weight, BMI, waist circumference
Morais et al., 2009 **A**	Brazil	single-blind, controlled	40	14/26	dyslipidemic without statin treatment	48.6	NA	yerba mate infusion	NA	20-days	1 L of yerba mate infusion	TC, LDL, HDL, TG, body weight, BMI, waist circumference
Morais et al., 2009 **B**	Brazil	single-blind, controlled	47	25/22	dyslipidemic without statin treatment	48.6	NA	yerba mate infusion	NA	40-days	1 L of yerba mate infusion	TC, LDL, HDL, TG, body weight, BMI, waist circumference
Morais et al., 2009 **C**	Brazil	single-blind, controlled	36	14/22	dyslipidemic without statin treatment	48.6	NA	yerba mate infusion	NA	60-days	1 L of yerba mate infusion	TC, LDL, HDL, TG, body weight, BMI, waist circumference
Morais et al., 2009 **D**	Brazil	single-blind, controlled	27	11/16	dyslipidemic without statin treatment	48.6	NA	yerba mate infusion	NA	90-days	1 L of yerba mate infusion	TC, LDL, HDL, TG, body weight, BMI, waist circumference
Morais et al., 2009 **E**	Brazil	single-blind, controlled	18	10/8	dyslipidemic with statin treatment	55.5	NA	yerba mate infusion	NA	20-days	1 L of yerba mate infusion	TC, LDL, HDL, TG, body weight, BMI, waist circumference
Morais et al., 2009 **F**	Brazil	single-blind, controlled	18	10/8	dyslipidemic with statin treatment	55.5	NA	yerba mate infusion	NA	40-days	1 L of yerba mate infusion	TC, LDL, HDL, TG, body weight, BMI, waist circumference
Morais et al., 2009 **G**	Brazil	single-blind, controlled	18	10/8	dyslipidemic with statin treatment	55.5	NA	yerba mate infusion	NA	60-days	1 L of yerba mate infusion	TC, LDL, HDL, TG, body weight, BMI, waist circumference
Morais et al., 2009 **H**	Brazil	single-blind, controlled	18	10/8	dyslipidemic with statin treatment	55.5	NA	yerba mate infusion	NA	90-days	1 L of yerba mate infusion	TC, LDL, HDL, TG, body weight, BMI, waist circumference
Pontin, 2010	Brazil	randomized, controlled	141	72/69	dyslipidemic and overweight	50.0	60/81	yerba mate tea	apple tea	8-weeks	1 L of yerba mate tea	TC, LDL, HDL, TG, body weight, BMI
Salerno, 2007	Brazil	non-randomized	19	10/9.	ischemic cardiopathy	64.1	9/10.	yerba mate infusion	water	5-days	500 mL yerba mate infusion	TC, LDL, HDL, TG, BMI

BMI – body mass index; **DBP** – diastolic blood pressure; **HDL** – HDL-cholesterol; **LDL** – LDL-cholesterol; **NA** – not available; **SBP** – systolic blood pressure; **TC** – total cholesterol; **TG** – triglycerides.

FIGURE 2 - Results of risk of bias assessment regarding the methodological quality of included studies: **A)** Risk of bias summary: review author's judgments about each risk of bias item for each included study; **B)** Risk of bias graph: review author's judgments about each risk of bias item presented as percentages across all included studies.

Effects of *I. paraguariensis* consumption on anthropometric measurements, lipid profile and blood pressure

Several previous reports have suggested that *I. paraguariensis* may possess anti-obesity properties, together with the potential to ameliorate the blood lipid profile of patients (Boaventura *et al.*, 2012; Gambero, Ribeiro, 2015; Kim *et al.*, 2015). In contrast, *I. paraguariensis* consumption may cause an increase in blood pressure due to the high concentration in caffeine (Cardozo Junior, Morand, 2016; Pontilho *et al.*, 2015). In this sense, the outcomes considered in this meta-analysis include some anthropometric measurements (body weight, BMI, and waist circumference), lipid profile and blood pressure of the participants in the clinical trials.

The lipid profile comprises the total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL) and LDL-cholesterol (LDL). In the case of the blood pressure, the values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) were both considered in the statistical analysis.

Figure 3 represents the forest plots resulting from the meta-analysis for the considered outcomes. Table II summarizes the meta-analysis results. Overall, it is possible to verify that *I. paraguariensis* consumption significantly reduces the body weight (Figure 3A) (WMD: -0.521 kg; 95% CI: -0.870 to -0.172; *p*-value= 0.003) and consequently the BMI (Figure 3B) of patients (WMD: -0.208 kg/m^2; 95% CI: -0.342 to -0.073; *p*-value= 0.002), which together with the significant reduction of waist circumference (Figure 3C) (WMD: -0.668 cm; 95% CI: -1.316 to -0.021; *p*-value= 0.043) demonstrate that, in fact, *I. paraguariensis* has some potential to treat obesity, which is in agreement with the findings of some clinical trials included in the present meta-analysis. It should be noted that no heterogeneity (I^2=0%) was observed among the trials for these outcomes (Table II). Concerning the results of TSA, it is possible to conclude by the visual inspection of the plots (Figure 4), that, in general, the cumulative *Z*-curve crosses the conventional significance test boundary and RIS-adjusted boundary value, indicating that the evidence needed to reach a conclusion was sufficient and no further trials were needed; thus, additional trials are not required and are unlikely to alter
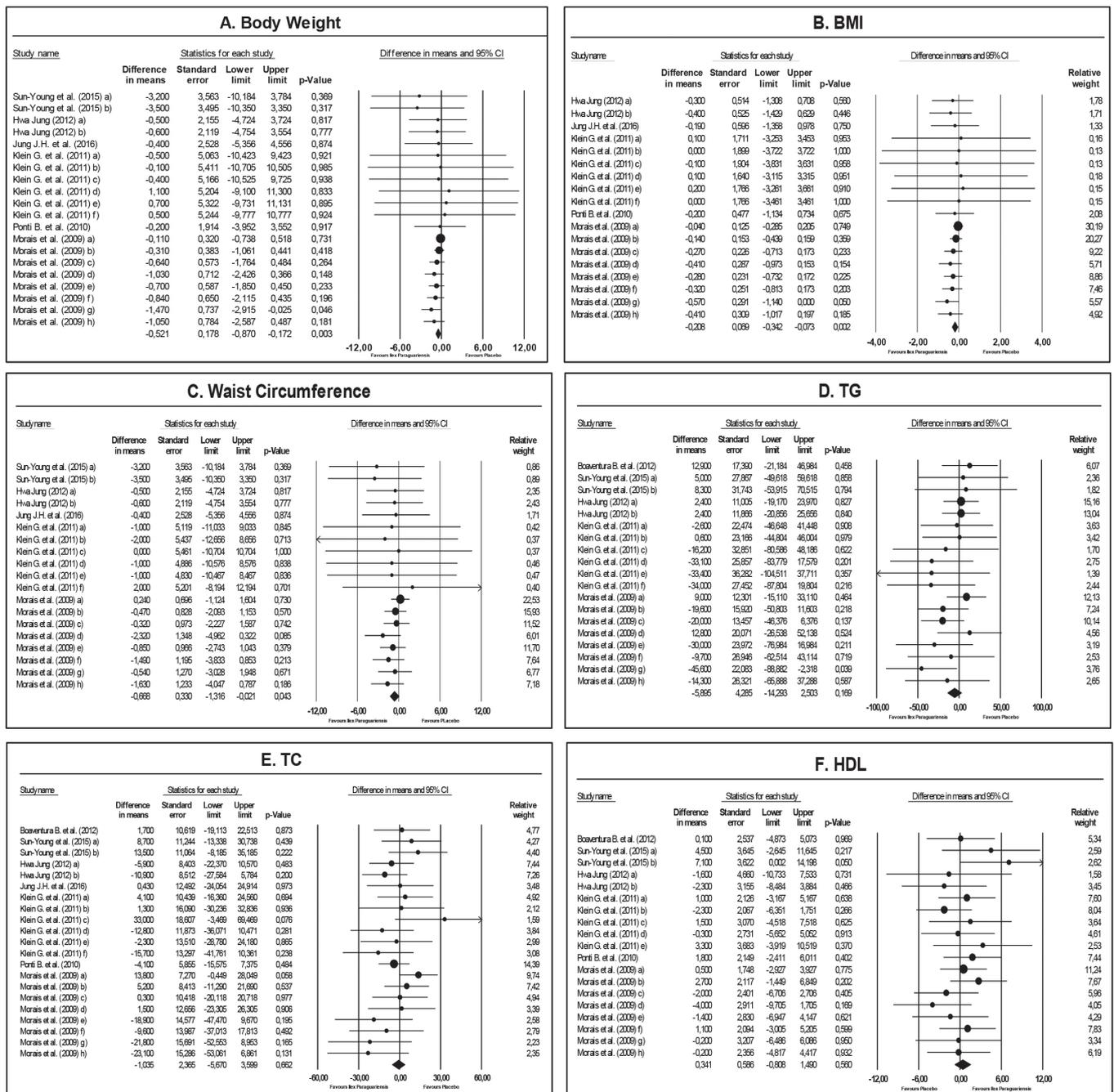
FIGURE 3 - Forest plots of comparisons of the effects *I. paraguariensis* consumption on the outcomes of this meta-analysis, compared with control arms. **BMI** – body mass index; **DBP** – diastolic blood pressure; **HDL** – HDL-cholesterol; **LDL** – LDL-cholesterol; **SBP** – systolic blood pressure; **TC** – total cholesterol; **TG** – triglycerides.

the conclusions obtained in the present work in what concerns the anthropometric measurements.

Regarding the remaining outcomes under analysis, it was verified that *I. paraguariensis* have no significant implications in both the lipid profile (Figures 3D, 3E, 3F, 3G and Table II) and blood pressure of patients (Figures 3H, 3I and Table II). However, the number of clinical trials evaluating the effects of *I. paraguariensis* in

these outcomes is reduced, leading to some heterogeneity (Table II) in addition to extremely high values of RIS (Table II), not being reached, indicating that more clinical trials are needed in order to achieve sufficient evidence to reach a conclusion.

Moreover, the TSA program was not able to produce the plots for the remaining outcomes due to the scarcity of data.
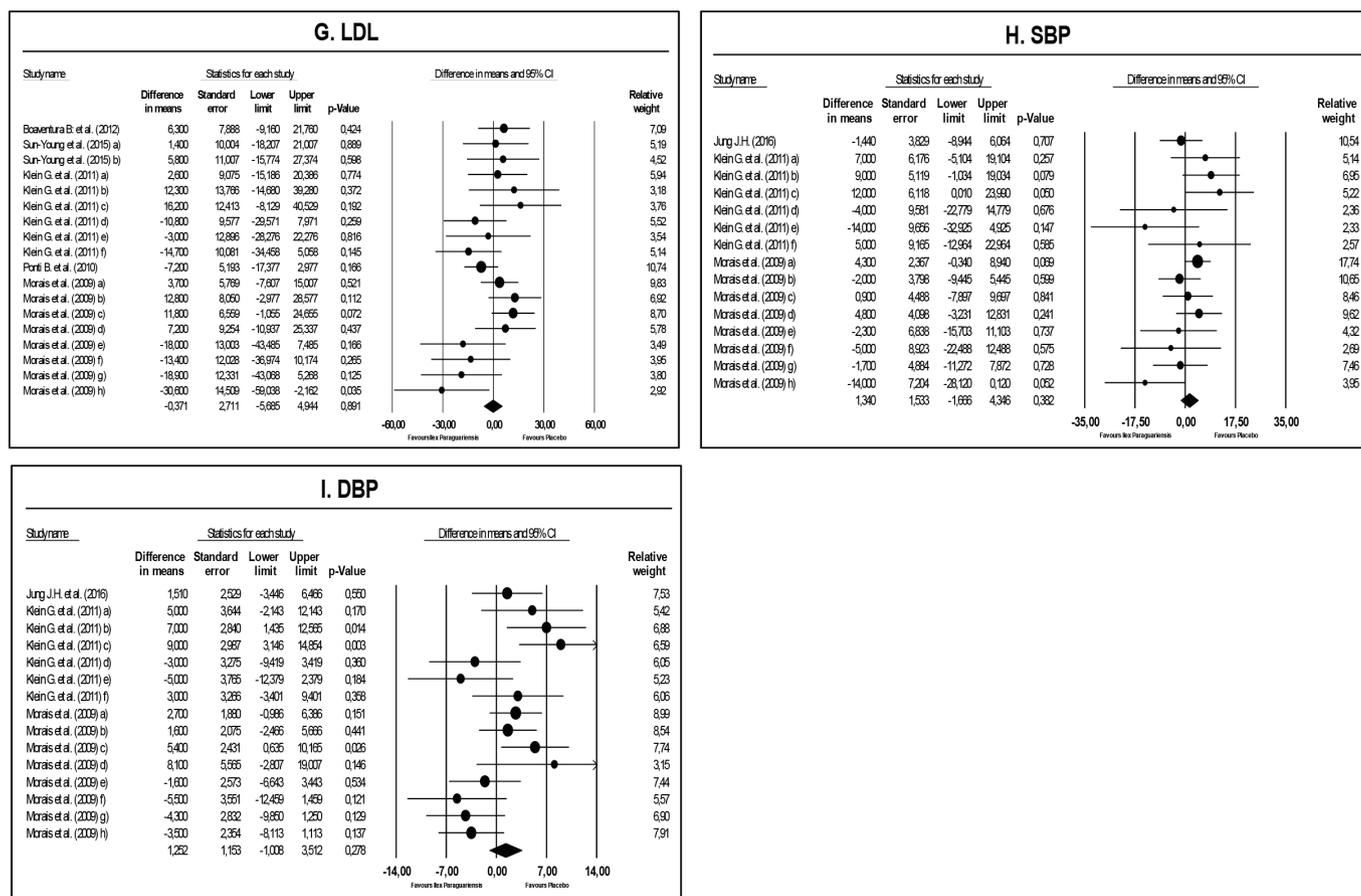
G. LDL

Study name	Difference in means	Standard error	Lower limit	Upper limit	p-Value	Relative weight
Boaventura B. et al. (2012)	6,300	7,888	-9,160	21,760	0,424	7,09
Sun-Young et al. (2015) a)	1,400	10,004	-18,207	21,007	0,889	5,19
Sun-Young et al. (2015) b)	5,800	11,007	-15,774	27,374	0,598	4,52
Klein G. et al. (2011) a)	2,600	9,075	-15,186	20,386	0,774	5,94
Klein G. et al. (2011) b)	12,300	13,766	-14,680	39,280	0,372	3,18
Klein G. et al. (2011) c)	16,200	12,413	-8,129	40,529	0,192	3,76
Klein G. et al. (2011) d)	-10,800	9,577	-29,571	7,971	0,259	5,52
Klein G. et al. (2011) e)	-3,000	12,896	-28,276	22,276	0,816	3,54
Klein G. et al. (2011) f)	-14,700	10,081	-34,458	5,058	0,145	5,14
Ponti B. et al. (2010)	-7,200	5,193	-17,377	2,977	0,166	10,74
Morais et al. (2009) a)	3,700	5,769	-7,607	15,007	0,521	9,83
Morais et al. (2009) b)	12,800	8,050	-2,977	28,577	0,112	6,92
Morais et al. (2009) c)	11,800	6,559	-1,055	24,655	0,072	8,70
Morais et al. (2009) d)	7,200	9,254	-10,937	25,337	0,437	5,78
Morais et al. (2009) e)	-18,000	13,003	-43,485	7,485	0,166	3,49
Morais et al. (2009) f)	-13,400	12,028	-36,974	10,174	0,265	3,95
Morais et al. (2009) g)	-18,900	12,331	-43,068	5,268	0,125	3,80
Morais et al. (2009) h)	-30,600	14,509	-59,038	-2,162	0,035	2,92
	-0,371	2,711	-5,685	4,944	0,891	

Difference in means and 95% CI — axis: -60,00 | -30,00 | 0,00 | 30,00 | 60,00
Favours Ilex Paraguariensis | Favours Placebo

H. SBP

Study name	Difference in means	Standard error	Lower limit	Upper limit	p-Value	Relative weight
Jung J.H. (2016)	-1,440	3,829	-8,944	6,064	0,707	10,54
Klein G. et al. (2011) a)	7,000	6,176	-5,104	19,104	0,257	5,14
Klein G. et al. (2011) b)	9,000	5,119	-1,034	19,034	0,079	6,95
Klein G. et al. (2011) c)	12,000	6,118	0,010	23,990	0,050	5,22
Klein G. et al. (2011) d)	-4,000	9,581	-22,779	14,779	0,676	2,36
Klein G. et al. (2011) e)	-14,000	9,656	-32,925	4,925	0,147	2,33
Klein G. et al. (2011) f)	5,000	9,165	-12,964	22,964	0,585	2,57
Morais et al. (2009) a)	4,300	2,357	-0,340	8,940	0,069	17,74
Morais et al. (2009) b)	-2,000	3,798	-9,445	5,445	0,599	10,65
Morais et al. (2009) c)	0,900	4,488	-7,897	9,697	0,841	8,46
Morais et al. (2009) d)	4,800	4,098	-3,231	12,831	0,241	9,62
Morais et al. (2009) e)	-2,300	6,838	-15,703	11,103	0,737	4,32
Morais et al. (2009) f)	-5,000	8,923	-22,488	12,488	0,575	2,69
Morais et al. (2009) g)	-1,700	4,884	-11,272	7,872	0,728	7,46
Morais et al. (2009) h)	-14,000	7,204	-28,120	0,120	0,052	3,95
	1,340	1,533	-1,666	4,346	0,382	

Difference in means and 95% CI — axis: -35,00 | -17,50 | 0,00 | 17,50 | 35,00
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I. DBP

Study name	Difference in means	Standard error	Lower limit	Upper limit	p-Value	Relative weight
Jung J.H. et al. (2016)	1,510	2,529	-3,446	6,466	0,550	7,53
Klein G. et al. (2011) a)	5,000	3,644	-2,143	12,143	0,170	5,42
Klein G. et al. (2011) b)	7,000	2,840	1,435	12,565	0,014	6,88
Klein G. et al. (2011) c)	9,000	2,987	3,146	14,854	0,003	6,59
Klein G. et al. (2011) d)	-3,000	3,275	-9,419	3,419	0,360	6,05
Klein G. et al. (2011) e)	-5,000	3,765	-12,379	2,379	0,184	5,23
Klein G. et al. (2011) f)	3,000	3,266	-3,401	9,401	0,358	6,06
Morais et al. (2009) a)	2,700	1,880	-0,986	6,386	0,151	8,99
Morais et al. (2009) b)	1,600	2,075	-2,466	5,666	0,441	8,54
Morais et al. (2009) c)	5,400	2,431	0,635	10,165	0,026	7,74
Morais et al. (2009) d)	8,100	5,565	-2,807	19,007	0,146	3,15
Morais et al. (2009) e)	-1,600	2,573	-6,643	3,443	0,534	7,44
Morais et al. (2009) f)	-5,500	3,551	-12,459	1,459	0,121	5,57
Morais et al. (2009) g)	-4,300	2,832	-9,850	1,250	0,129	6,90
Morais et al. (2009) h)	-3,500	2,354	-8,113	1,113	0,137	7,91
	1,252	1,153	-1,008	3,512	0,278	

Difference in means and 95% CI — axis: -14,00 | -7,00 | 0,00 | 7,00 | 14,00
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FIGURE 3 (cont.) - Forest plots of comparisons of the effects *I. paraguariensis* consumption on the outcomes of this meta-analysis, compared with control arms. **BMI** – body mass index; **DBP** – diastolic blood pressure; **HDL** – HDL-cholesterol; **LDL** – LDL-cholesterol; **SBP** – systolic blood pressure; **TC** – total cholesterol; **TG** – triglycerides.

The sensitivity analysis was also performed by excluding one or more studies from the analysis to see how this affected the results. The results showed that the pooled effects of *I. paraguariensis* consumption on the considered outcomes did not change substantially if a single or a few studies were omitted (data not shown).

Overall, the sensitivity analysis demonstrated that the findings of this meta-analysis on the considered outcomes are robust.

During this systematic review, it was verified that *I. paraguariensis* was administered to the patients at different intervention durations. Therefore, a meta-regression analysis (Figure 5) was conducted to evaluate the association between changes in body weight, BMI and waist circumference, and the study duration. The results of meta-regression suggest no significant correlation between the intervention duration and body weight (Figure 5A; *p*-value= 0.0896), BMI (Figure 5B; *p*-value= 0.105) or waist circumference (Figure 5C; *p*-value= 0.125).

In summary, the findings clearly showed that the use of *I. paraguariensis* might be useful against obesity.

In the previous comprehensive reviews published by Bracesco *et al*., 2011 and by Gambero and Ribeiro, 2015, some possible mechanisms of action, regarding the anti-obesity potential of *I. paraguariensis*, were addressed; however, more studies at cellular and molecular level must be undertaken to better understand this biological activity of *I. paraguariensis*.

Publication bias

To analyse the publication bias, funnel plots were generated for the defined outcomes considering the Trim and Fill adjustment (Figure 6). These funnel plots included both the observed studies (shown as blue circles) and the necessary imputed studies (shown as red filled circles) to obtain the absence of bias. It was observed that there is an absence of bias for body weight (Figure 6 A). For the remaining outcomes, some studies are imputed on the

TABLE II - Meta-analysis results of the effects of *I. paraguariensis* consumption on the considered outcomes

Outcomes	Results	Number of trials	95% CI	*p*-value	I² (%)	RIS
Body Weight	Total	20	-	-	-	579
	WMD observed	-	-0.521 (-0.870; -0.172)	0.003*	0	
	WMD adjusted	-	-0.521 (-0.870; -0.170)	-	-	
BMI	Total	19	-	-	-	499
	WMD observed	-	-0.208 (-0.342; -0.073)	0.002*	0	
	WMD adjusted	-	-0.209 (-0.343; -0.075)	-	-	
Waist Circumference	Total	19	-	-	-	973
	WMD observed	-	-0.668 (-1.316; -0.021)	0.043*	0	
	WMD adjusted	-	-0.504 (-1.127; 0.119)	-	-	
TG	Total	10	-	-	-	2334
	WMD observed	-	-5.895 (-14.293; 2.503)	0.169	0	
	WMD adjusted	-	-1.249 (-9.540; 7.042)	-	-	
TC	Total	21	-	-	-	19353
	WMD observed	-	-1.305 (-5.670; 3.599)	0.662	3.869	
	WMD adjusted	-	-0.001 (-4.849; 4.829)	-	-	
HDL	Total	19	-	-	-	64545
	WMD observed	-	0.341 (-0.808; 1.490)	0.560	0	
	WMD adjusted	-	0.341 (-0.808; 1.490)	-	-	
LDL	Total	18	-	-	-	1436902
	WMD observed	-	-0.371 (-5.685; 4.944)	0.891	33.670	
	WMD adjusted	-	1.274 (-4.369; 6.918)	-	-	
SBP	Total	15	-	-	-	4561
	WMD observed	-	1.340 (-1.667; 4.346)	0.382	23.509	
	WMD adjusted	-	2.621 (-0.725; 5.966)	-	-	
DBP	Total	15	-	-	-	2421
	WMD observed	-	1.252 (-1.008; 3.512)	0.278	0	
	WMD adjusted	-	1.252 (-1.008; 3.512)	-	-	

BMI – body mass index; **CI** – confidence interval; **DBP** – diastolic blood pressure; **HDL** – HDL-cholesterol; **LDL** – LDL-cholesterol; **RIS** – required information size; **SBP** – systolic blood pressure; **TC** – total cholesterol; **TG** – triglycerides; **WMD** – weighted mean differences; ***** - indicates a significant result.

right or on the left size to "adjust" the funnel plots for the absence of publication bias. Therefore, by observing the funnel plots, it is possible to verify that publication bias cannot be completely excluded. The values of WMD adjusted for the outcomes under analysis are presented in Table II.

In addition to the visual inspection of the funnel plots, the presence of publication bias was explored using Egger's regression test. This test did not indicate evidence of publication bias for the impact of *I. paraguariensis* consumption on the evaluated outcomes (Table III).

CONCLUSIONS

In conclusion, this systematic review with meta-analysis and TSA of clinical trials, demonstrated the positive effects of *I. paraguariensis* consumption on the reduction of body weight, BMI, and waist circumference of patients, showing its anti-obesity potential. However, special attention must be given due to the high concentrations of caffeine found in this plant. Future clinical trials should evaluate the effects of
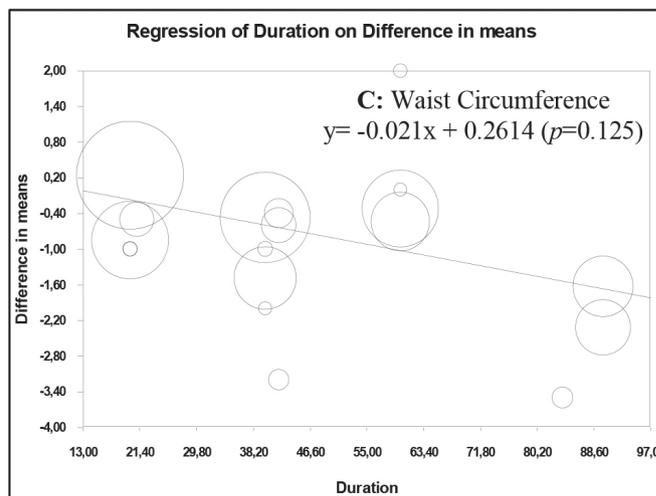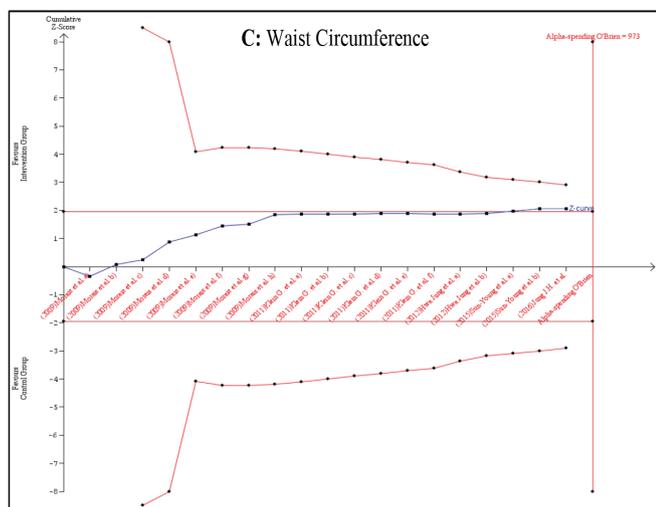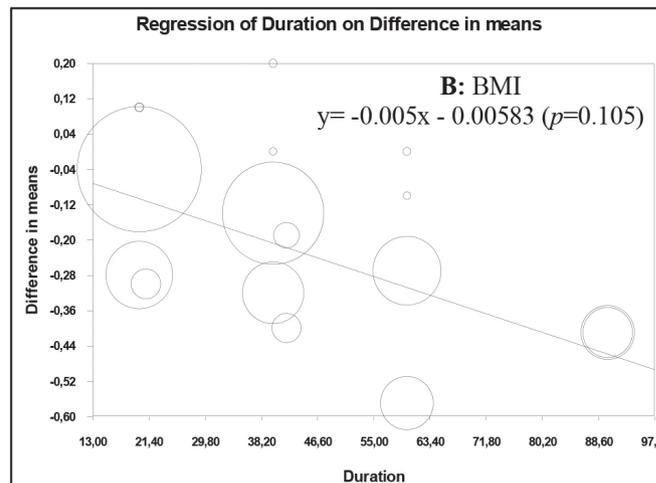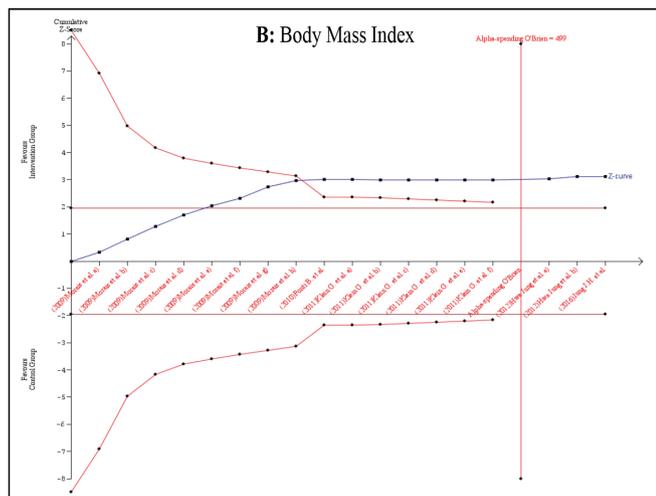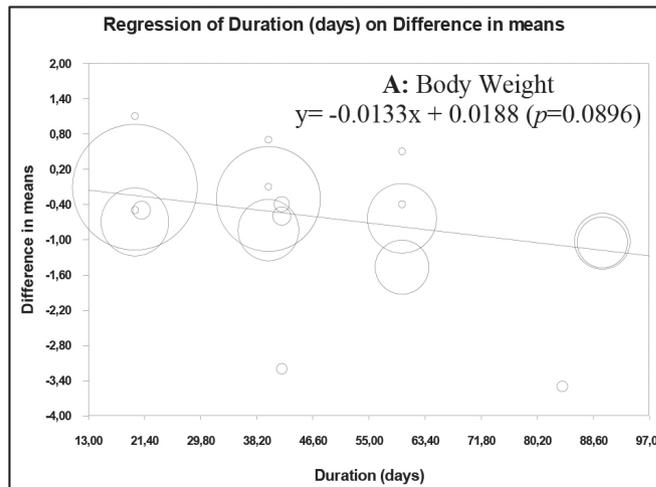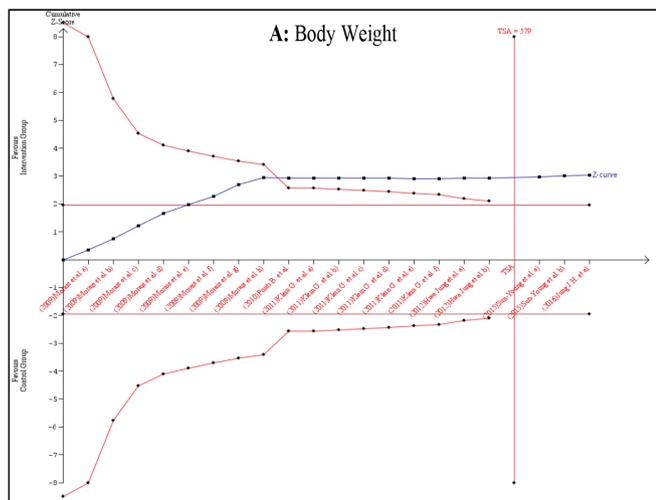
FIGURE 4 - Trial Sequential Analysis (TSA) on pooled results of the effects of *I. paraguariensis* consumption on the anthropometric measurements considered in this meta-analysis.

FIGURE 5 - Meta-regression plots of the association between calculated weighted mean differences (WMD) in body weight, body mass index (BMI) and waist circumference, and the study duration.

TABLE III - Assessment of publication bias for the impact of *I. paraguariensis* consumption on the considered outcomes

Outcomes	Egger's regression test			
	95% CI	*t*	df	*p*-value
Body weight	-0.650; 0.130	1.390	18	0.182
BMI	-0.655; 0.231	1.013	16	0.326
Waist circumference	-0.824; 0.151	1.455	17	0.164
TG	-2.126; 0.135	1.857	17	0.080
TC	-2.161; 1.063	0.712	19	0.485
HDL	-1.460; 2.233	0.441	17	0.664
LDL	-3.015; 0.975	1.084	16	0.295
SBP	-2.537; 0.574	1.360	13	0.196
DBP	-3.930; 3.630	0.086	13	0.933

BMI – body mass index; **CI** – confidence interval; **DBP** – diastolic blood pressure; **df** – degrees of freedom; **HDL** – HDL-cholesterol; **LDL** – LDL-cholesterol; **SBP** – systolic blood pressure; **TC** – total cholesterol; **TG** – triglycerides.
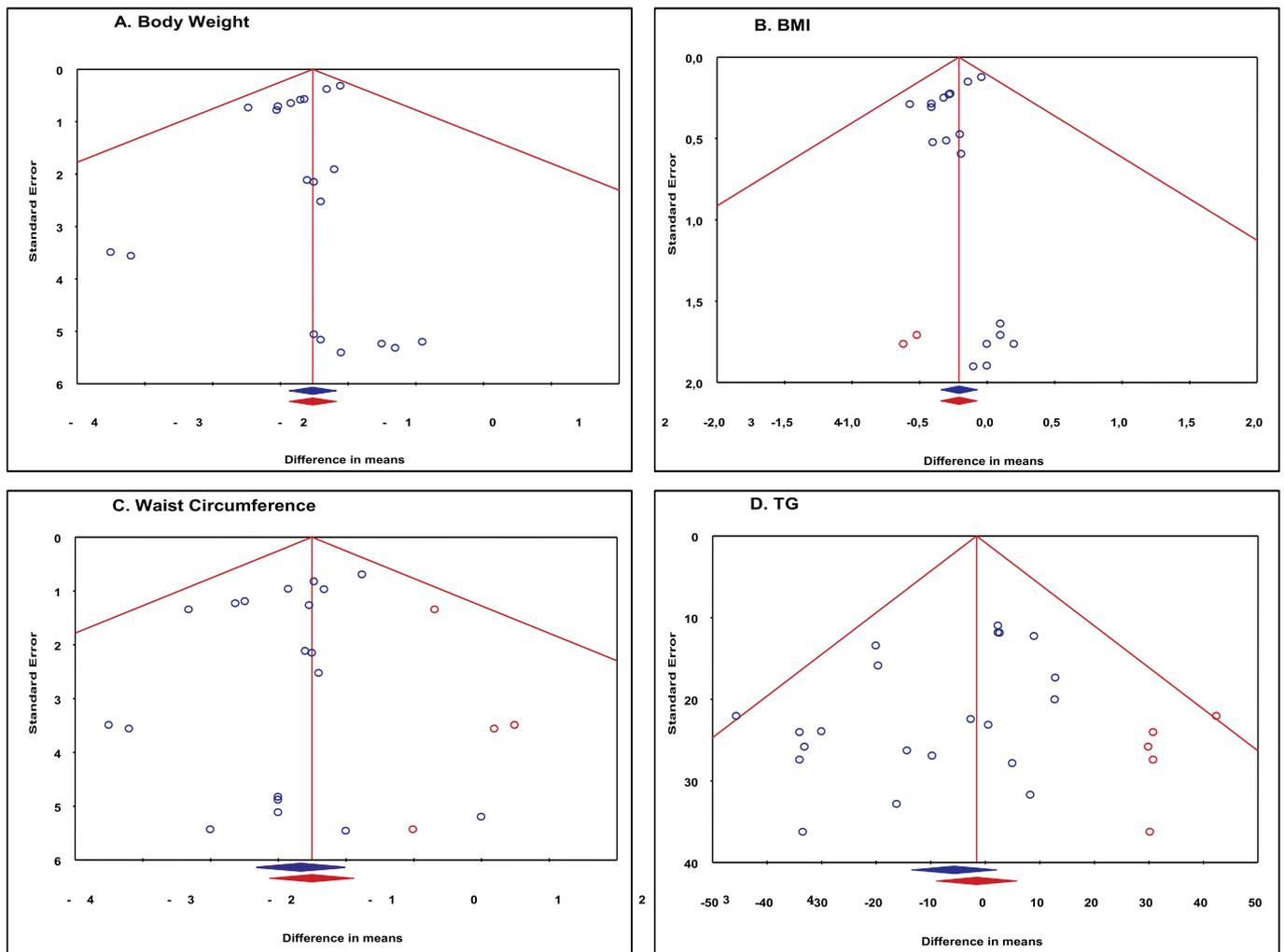
FIGURE 6 - Funnel plots of standard error by difference in means (publication bias tests) of the effects of *I. paraguariensis* consumption on the outcomes of this meta-analysis. **BMI** – body mass index; **DBP** – diastolic blood pressure; **HDL** – HDL-cholesterol; **LDL** – LDL-cholesterol; **SBP** – systolic blood pressure; **TC** – total cholesterol; **TG** – triglycerides.
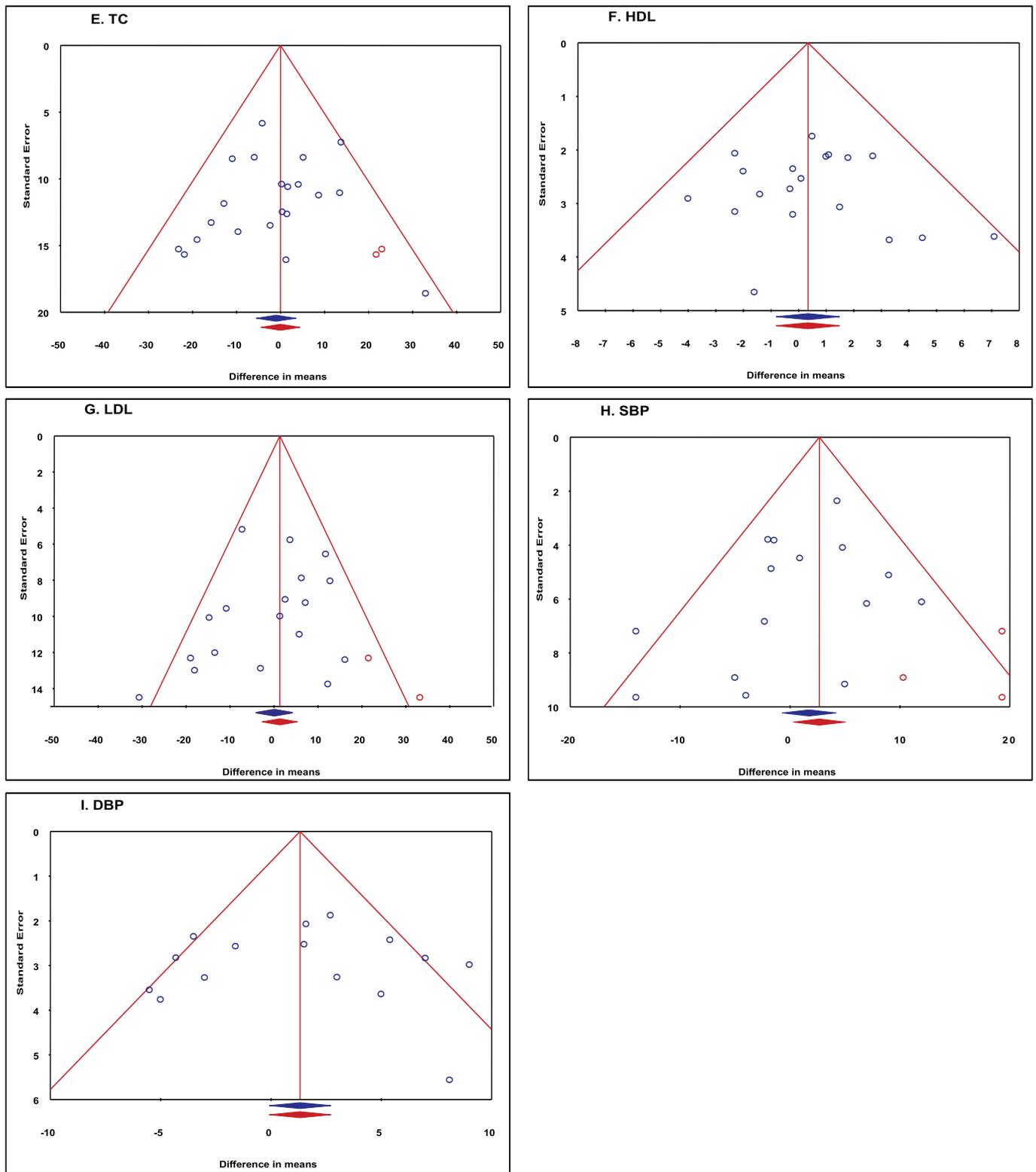
FIGURE 6 (cont.) - Funnel plots of standard error by difference in means (publication bias tests) of the effects of *I. paraguariensis* consumption on the outcomes of this meta-analysis. **BMI** – body mass index; **DBP** – diastolic blood pressure; **HDL** – HDL-cholesterol; **LDL** – LDL-cholesterol; **SBP** – systolic blood pressure; **TC** – total cholesterol; **TG** – triglycerides.

I. paraguariensis on lipid profile and blood pressure of patients, together with the evaluation of its consumption by healthy people.

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