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Metabolic profile and atherogenic indices of rats treated with *Tamarindus indica* and *Menthapiperita* juice.

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Abstract

Changes in diet, physical inactivity and stress contribute to obesity, cardiovascular disease and atherosclerosis. Literature shows that bioactive compounds may result in benefits to human health. The aim of this study was to evaluate the glycemic, lipid profile and atherogenic indices of Wistar rats treated with *Menthapiperita* and *Tamarindus indica* juice. Animals received food and water *ad libitum* and were divided into 3 groups: control group (G1) that received 0.5 mL of water twice daily; *Mentha*group (G2) treated with 0.5 mL of mint juice and group treated with *Tamarindus indica* juice (G3), that received 0.5 mL of tamarind juice twice daily for 40 days. Groups G1, G2 and G3 received the drinks by intragastric rout. After this period the animals were sedated with sodium pentobarbital for blood collection and evaluation of the biochemical profile: total cholesterol, HDL-c, LDL-c, triglycerides and glucose. Atherogenic indexes were also calculated. The results showed a reduction of total cholesterol, LDL-c, triglyceride, body weight and atherogenic indices; and increase in the levels of HDL-c. We may suggest that the use of mint and tamarind juice can positively affect the biochemical parameters and reduce the atherogenic indexes of Wistar rats.

Keywords: *Tamarindus indica, Mentha piperita,* glycemia, cholesterol, triglycerides, HDL-c.

Introduction

Eating habits and a sedentary lifestyle in modern societies are associated to a number of risk factors that favor the development of diabetes, metabolic syndrome and cardiovascular diseases (CVD) that are the main causes of death worldwide. As a result, much research based on diet - health binomial have been developed.This worrying scenariohas led to the development of several studies with therapeutic alternatives since conventional medications are normally expensive and are related to numerous side effects[1-4].

Menthapiperita L (Labiatae family) is widely used in Brazil and other countries with several purposes such as treatment of loss of appetite, common cold, bronchitis, fever, nausea, vomiting, antioxidants and antimicrobial activities. Studies have shown that the mint extracts decrease glucose levels, total cholesterol, triglycerides, VLDL-c and LDL-c levels in diabetic rats[5-8].

The *Tamarindus indica* L. (Fabaceae family) has numerous medicinal properties, with an emphasis on antioxidant, lipid-lowering, hypoglycemic, anti-inflammatory effects. In addition it may perform antimicrobial effect and control of satiety, thus it plays a potential role in the treatment or prevention of obesity and other chronic associated diseases [9-14].

Because of the many effects of *Menthapiperita* and *Tamarindus indica* health, the aim of this study was to compare the effect of these plants on the biochemical profile and atherogenic indices of Wistar rats.

Methods

Menthapiperita and *Tamarindus indica* juice

Mentha leaves were mashed in water (100 g/L) for 3 minutes. The pulp of *Tamarindus indica*was also mashed in water (100 g/L) for 3 minutes.

Group of Animals

The experiment was approved by the Animal Research Ethics Committee of the UniversidadeMetodista de Piracicaba (UNIMEP, Marília, SP, Brazil) under protocol number 2500000765/07-47. Animals weighing approximately 310g to 400g, were kept in the vivarium at UNIMEP (Lins City *Campus*) in collective cages under a dark/light cycle of 12 hours, room temperature of 22 ± 2 C, and relative air humidity of 60 ± 5%. During the experimental period, the

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animals received water and food *ad libitum* and were cared for according to the recommendations of the Canadian Council's "Guide for the care and use of experimental animals".

After a period of 10 days of acclimation to laboratory conditions, the animals were divided randomly in the experimental groups, which were identified according to the treatment they would receive:

G1: control group that received water and rat feed*ad libitum,*andwaterusing intragastricroute twice a day (in the morning and late afternoon);

G2: group that received water and rat feed *ad libitum,* and *Menthapiperita* juice using intragastric route twice a day (in the morning and late afternoon).

G3: group that received water and rat feed *ad libitum,* and *Tamarindus indica* juice using intragastricroute twice a day (in the morning and late afternoon).

The treated groups received a dose of 0.29 g/Kg twice a day (similar to the popular consume of these plants: a man weighing 70 kg consuming 200 mL of the juice twice a day).

After a period of 40 days, the animals were euthanized with a lethal intraperitoneal injection of 200 mg/Kg of thiopental. After death, blood samples were drawn from the vena cava to determine the biochemical profile: glycaemia, total cholesterol (TC), High Density Lipoprotein (HDL-c), Low Density Lipoprotein (LDL-c), and triglycerides (TG). Non-HDL-c were calculated (Total Cholesterol - HDL-c). The glucose and lipid levels were measured in mg/dL. Atherogenic Index (AI), Atherogenic Coefficient (AC), Cardiac Risk Ratio 1 (CRR1)and Cardiac Risk Ratio 2 (CRR2) were evaluated after Ahmadvand et al. [15]; Munshi, Joshi, Rane [16]; Ikewuchi [17]: AI = log (TG/HDL-c); AC = (TC – HDL-c)/HDL-c; CCR1 = TC/HDL-c and CCR2 = LDL-c/HDL-c [15-17].

Weight was performed at the 1 , 8 , 15 , 23 , 31 and 40 days.

Statistics

ANOVA and Tukey Test were usedfor the statistical analysis and the variables were presented as mean and standard error mean, adopting a 5% level of significance.

Results

Table 1 shows that animals in the three experimental groups showed similarity mean weight at the start of treatment. After the treatment period, a significant decrease in weight gain of animals treated with mint juice (G2) and tamarind juice (G3) when compared to G1 (control group).

Table 1. Weight of the animals of control group (G1), group treated with *M. piperita* (G2) and group treated with tamarind (G3).

Parameter	G1	G2	G3
Weight at the beginning (g)	343.3 ± 12.9A	337.8 ± 28.0A	348.7 ± 24.A
Weight at the end (g)	392.6 ± 23.3C[1]	358.4 ± 14.7A	389.6 ± 20.9B

[1]Different letters indicate a significant difference between the treatments at a level of 5%.

Animals treated with mint showed decrease in the levels of glucose, cholesterol, LDL-c and increase in HDL-c (Table 2). Animals treated with tamarind showed improvement in total cholesterol, LDL-c, HDL-c and non-HDL-c. When we compare G2 and G3, we may see that lower levels of glucose and LDL-c in the group treated with mint.

Table 2. Biochemical parameters of the animals of control group (G1), group treated with *M. piperita* (G2) and group treated with tamarind (G3).

Parameter	G1	G2	G3
Glucose	132.6 ± 18.5 A	110.6 ± 22.4 B	131.6 ± 28.1 A
Total Cholesterol	93.4 ± 17.5 A	70.7 ± 13.0 B	67.9 ± 16.4 B
HDL-c	38.7 ± 16.8 A	59.3 ± 11.8 B	52.1 ± 15.4 B
Triglycerides	120.3 ± 47.6 A	133.6 ± 54.1 A	126.1 ± 82.1 A
LDL-c	58.2 ± 8.5 A	28.6 ± 6.5 C	35.8 ± 12.3 B
Non-HDL-c	56.08 ± 13.6 B	11.89± 9.1 A	15.67± 12.8 A

[1]Different letters indicate a significant difference between the treatments at a level of 5%. HDL-c: High density lipoprotein; LDL-c: Low density lipoprotein; Non-HDL-c: non-HDL- cholesterol.

Results for the Atherogenic Index (AI), Atherogenic Coefficient (AC), Cardiac Risk Ratio 1 (CCR1) and Cardiac Risk Ratio 2 (CCR2) show that that both treatments promoted significant reduction (Table 3).

Table 3. Atherogenic Index (AI), Atherogenic Coefficient (AC), Cardiac Risk Ratio 1 (CCR1) and Cardiac Risk Ratio 2 (CCR2) of the animals of control group (G1), group treated with *M. piperita* (G2) and group treated with tamarind (G3).

Parameter	G1	G2	G3
AC	1.481± 0.26 A	0.208± 0.07 B	0.307± 0.05 C
AI	0.506± 0.29 A	0.347± 0.06 B	0.382± 0.19 B
CCR1	2.49± 0.46 A	1.18± 0.27 B	1.28± 0.34 B
CCR2	1.51± 0.33 A	0.47± 0.17 B	0.67± 0.14 B

[1]Different letters indicate a significant difference between the treatments at a level of 5%.

Discussion

The use of plants and other natural products rich in bioactive substances, is very common among population worldwide. Herbs and spices are an excellent source of antioxidants that reduce the impact of oxidative modification of LDL-c and consequent development of atherosclerosis and many other degenerative diseases. Associated with these pathologies is the Metabolic Syndrome that is characterized by a cluster of risk factors, which cause inflammatory and metabolic alterations that increase vascular risk and may promote the development of atherosclerosis [18-20].

Menthapiperita in aqueous solution brings together numerous health benefits associated with the phytochemical content, mainly flavonoids and phenolic acids present in the plant leaf. Some of the compounds are menthol, menthone caffeic acid, acetaldehyde, menthyl esters, limonene, pinene, cardial glycosides, phellandrene, cadinene, pugelone, and dimethyl sulfide; traçosconstituintesincluem alpha-pinene, sabinene, terpinolene, ocimene, diterpenes, gamma-terpinene, steroids, fenchene, alpha and beta-thujone, coumarin, citronellol, carotenes, tocopherols, betaine, choline, saponin, and tannins [4-5, 21-22].

Pharmacological studies have shown that *M. piperita* has antioxidant activity, reducing the effects of the free radicals that are related to diseases such as cancer and atherosclerosis. These properties are due to the presence of bioactive compounds [23-24].

Sharafi et al. [25] evaluated the protective activity of bioactive compounds in the essential oil of *M.piperita*and showed similar results to our study. Barbalho et al. [26] studied the effects of *M. piperita*tea and juice in animals fed a hypercaloric diet and found positive effects of both solutions in lipid profile and observed substantial reduction in food intake and in percentage of weight gain. Barbalho et al. [7] showed that this species of mint may produce a significant reduction in glucose, cholesterol, LDL-c and triglycerides levels as well as significant increase in HDL-c levels in diabetic rats, contributing with the prevention of diabetes and dyslipidemia.

Figueroa-Perez et al.[5] showed that the saponins and alkaloids present in the *M. piperita*leaf exert anti-hyperglycemic and hypolipidemic effects through some mechanisms such as regulation of insulin secretion, inhibition of glucose absorption in the intestinal lumen and decreased accumulation of lipids in the liver.

Johari et al. [22] evaluated the extract of *M. piperita* in rats and postulate that compounds as taurine, betaine, alanine, glycine and L-leucine are related to the hypocholesterolemic effects.

Several studies have linked the use of the pulp of *Tamarindus indica* with hypolipidemic action, showing reduction of total cholesterol, LDL-c and increasedHDL-c. It may also exhibit anti-hyperglycemic and antioxidant properties and significant decrease in body weight in animal models thus could prevent the occurrence of cardiovascular risk factors[9, 11-12, 27-32].

Sasidharan et al. [33] investigated the effects of *T. indica* pulp aqueous extract in diet-induced obese Sprague-Dawley rats and showed decrease in the levels of leptin, triglyceride, cholesterol, LDL-c, and increased in HDL-c. They also observed reduction of body weight and significant reduction in adipose tissue weights. The reduction of leptin and activity of Fatty Acid Synthase improve the efficiency of the antioxidant defense system.

T. indica presents hypocholesterolemic and antioxidant properties by increasing Apo-A1 (Apoliprotein A1) gene expression, and LDL-c receptor in the liver, by decreasing HMG-CoA reductase action and by inhibition of gene expression of MTP (Microsomal Triglyceride Transfer Protein). It also prevents oxidative damage to LDL-c cholesterol, which is an important risk factor for atherosclerosis development [29, 34].

According to several studies, this fruit has high antioxidant capacity and is rich in organic acids, pectin, vitamins, mineral content, polyphenols and flavonoids. The crude extract of tamarind pulp has antioxidant phenolic compounds that enhance the efficiency of the antioxidant defense system. The presence of phenolic antioxidants as pro-anthocyanidins that are capable of promoting health benefits enables the application of this plant in the pharmaceutical industry[9, 11- 13, 27-28, 35-36]. Sharma et al. [28] showed that the pectin extracted from *T. indica* pulp has antioxidant properties higher than other sources as apple, skin citrus pectin, commercial pectin, guar gum, oligosaccharides and xanthan.The antioxidant properties may be related to the improvement of the efficiency of superoxide dismutase, catalase and glutathione peroxidase [28, 37].

The atherogenic indices increase the risk of developing cardiovascular disease and low atherogenic indexes are protective against coronary heart disease. Oxidative stress and inflammation also increase the atherogenic indexes and vice-versa. Our study showed that animals treated with mint and tamarind present significant decrease of AC, CCR1 and CCR2, indicating that these plants reduce the risk factors for metabolic diseases possibly due to the presence of anti-oxidant and anti-inflammatory compounds [15-16, 38- 39].

In addition to the benefits that the studied plants promote on the metabolic profile, we should emphasized that they are easily accessible, have low cost and do not show toxicity. Besides, the richness in bioactive compounds could be used for prevention or slowing the progress of many chronic diseases associated with oxidative stressand inflammation [8, 12, 29, 40-41].

Conclusions

Our results show that the use of the juice of *Menthapiperita*improved glycaemia, total cholesterol, LDL-c and HDL-c. *Tamarindus indica* also showed benefits in total cholesterol, LDL-c and HDL-c. Both plants decreased the atherogenic index, coefficient index, and Cardiac risk 1 and 2 and may positively influence the body weight suggesting that they have potential to be used in the prevention of cardiovascular diseases. However, the use and the precise doses in human beings still need more detailed studies.

Conflict Of Interests

Authors declare no conflict of interests.

Authors Contributions

SMB and SCCT: conception and design of the manuscript. APPM, ELG and VSS: helped on the data collection and discussion. SMB and scct: wrote the paper.MO: performed the statistic analysis.

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