Effect of dietary plants on the production of immunoglobulins A in healthy Wistar rats

Efeito de plantas dietéticas na produção de imunoglobulinas A em ratos Wistar saudáveis

Flávia Imanishi Ruzon-Camargo¹, Eduardo Vignoto Fernandes², Mayara Bocchi³, David Michel de Oliveira⁴, Emerson José Venancio⁵, Raul Jorge Hernan Castro Gómez⁶

Abstract

Introduction: some plants such as turmeric, cinnamon, and okra are known to have therapeutic functions such as antioxidant and anti-inflammatory activity. Furthermore, an immunomodulatory role has been observed in the production of antibodies, in particular immunoglobulin A (IgA), which mediates a variety of protective functions for the organism. Objective: the aim of the present study was to investigate the effect of dietary plants on the production of IgA in healthy Wistar rats. Methods: thus, 48 male Wistar rats of 90 days of age were allocated to four groups. The animals were treated for 14 days with dried turmeric, cinnamon, or okra (50, 50, 12.5 mg/day, respectively) in phosphate buffered saline, or with only phosphate buffered saline by gavage. The animals received water and feed ad libitum. Body mass and relative weight of peritoneal fat, adrenal gland, kidney, spleen, liver and thymus, biochemical parameters, and IgA levels were analyzed. Results: no significant changes were observed in the body mass, relative weight of organs and tissues, and biochemical parameters. An increase in serum IgA levels was observed in animals treated with turmeric or cinnamon. Conclusion: we conclude that the treatment with turmeric and cinnamon increased IgA production. Therefore, our study supports the idea that dietary supplementation with these plants may improve humoral immunity.

Keywords: Curcuma; Cinnamomum; Abelmoschus; Antibodies; Medicinal plants.
Introduction

The use of plants and their parts as therapeutic agents is a common practice. Many have medicinal properties and their application, especially in traditional medicine, is well recognized and established. Although they have some aspects similar to modern drugs, in general, they are less aggressive and safer.

Turmeric (Curcuma longa), a member of the Zingiberaceae family, is an important spice, which confers color and aroma, in addition to demonstrating medicinal properties, such as antiviral, antioxidant, anti-inflammatory, antibacterial, and antifungal activity, thus presenting a potential for action against various diseases, such as diabetes, allergies, and arthritis. Furthermore, for Jagetia and Aggarwalm, this substance has been shown to be a potent immunomodulatory agent on T and B cells, macrophages, neutrophils, and natural killer and dendritic cells.

Okra (Abelmoschus esculentus), a member of the Malvaceae family, is valued for being a nutritious fruit and abundant in minerals. In traditional Chinese medicine, it is used to promote diuresis and to treat dental diseases and gastric irritation, as reported by NdjouenkeuetaL. Furthermore, it has been shown to be useful in the treatment of inflammation, constipation, and urinary retention.

Cinnamon is obtained from the bark of several species of the genus Cinnamomum, Lauraceae family, and is a highly appreciated seasoning for its flavor and health benefits. As a traditional medicinal plant, it is very popular in the treatment of various disorders such as chronic gastric disorders, arthritis, and the common cold. It has anti-ulcer, anti-inflammatory, antioxidant, antimicrobial, and anti-diabetic action and its consumption also provides trace amounts of choline, which helps in acetylcholine synthesis and fat metabolism.

The literature has shown that the addition of turmeric, cinnamon, and okra to the diet have positive effects on variables including body mass, blood glucose, lipid profile, and the immune system, such as increased production of immunoglobulin A (IgA). Serum IgA was first discovered in 1953 and mediates a variety of protective functions through interaction with FcRα receptors on monocytes...
Effect of dietary plants on the production of immunoglobulins A in healthy Wistar rats

As mentioned above, studies that assess the action of turmeric, cinnamon, and okra are generally carried out in animals with some type of disease, such as overweight and diabetes (16) or submitted to a diet rich in fructose, or with hyperlipidemia (6, 20). However, few studies have evaluated the effects of these plants on physiological parameters in healthy animals (17). Therefore, the aim of the present study was to verify the effect of dietary plants on the production of immunoglobulin A in healthy Wistar rats.

Methods

Sample preparation

Dried and powdered turmeric (Curcuma longa) and cinnamon (Cinnamomum spp.), as well as okra fruits (Abelmoschus esculentus) were purchased in the local market of the city of Londrina, Paraná state, Brazil. The okra fruits were sanitized with soap and water and then soaked in bleach (250 ppm L⁻¹) for 15 minutes, before being rinsed with running water. The excess water was removed before the fruits were chopped, dried in an oven at 40 °C for 24 hours, and milled in an analytical mill (A 11 basic®, IKA, Germany). All the products were sieved to obtain standardized samples with 100 mesh granulometry. All samples were packed in plastic flasks which were stored away from light in a dry and cool place until use.

Characterization of plant samples

The samples of cinnamon, turmeric, and okra were characterized according to their physical and chemical compositions. All analyses were performed in triplicate. The results are expressed as mean ± standard deviation. Determination of moisture, ash, protein, lipid, and carbohydrate content was performed according to Latimer (21).

Determination of antioxidants

The plant samples were extracted in water and ethanol solution (1:4) for 24 hours, at 2 °C. The mixture was centrifuged in a refrigerated centrifuge (5804 R®, Eppendorf, Germany) under the following conditions: 4 °C, 1000 x g, for 10 minutes. The extract obtained was used for the determination of antioxidants.

Total phenolic compounds were quantified using the Folin-Ciocalteau method (22). The absorbance reading was performed on a spectrophotometer (GENESYS 6®, Thermo Fisher Scientific, United States of America - USA) at 760 nm. The results were expressed based on a standard curve of gallic acid, in mg of gallic acid equivalent (GAE)/g of sample. The analysis was performed in triplicate and the values expressed as mean ± standard deviation.

The antioxidant capacity was determined using the 2,2'-diphenyl-1-picrilhydrazyl (DPPH) method. The extracts were placed in a mixture containing 0.1 M acetate buffer (pH 5.5) and an ethanol DPPH solution (250 µM in ethanol). After 15 minutes, the absorbances were read on a spectrophotometer (GENESYS 6®, Thermo Fisher Scientific, USA) at 517 nm. The antioxidant capacity was calculated in relation to the activity of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), based on a standard curve. The results were expressed as Trolox Equivalent Antioxidant Activity (TEAC) in µM Trolox/g of sample. The analysis was performed in triplicate and the values expressed as mean ± standard deviation.

The determination of antioxidant capacity was based on the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) method (ABTS). The extracts were placed in an ABTS solution in 20 mM phosphate
buffer (pH 7.4). The absorbance reading was performed in a spectrophotometer (GENESYS 6®, Thermo Fisher Scientific, USA) at 734 nm. The antioxidant capacity was calculated in relation to the Trolox activity, based on a standard curve. The results were expressed in TEAC in µM Trolox/g of sample. The analysis was performed in triplicate and the values expressed as mean ± standard deviation.

**Determination of curcumin content**

The curcumin content (%) of the Curcuma longa sample was determined by high performance liquid chromatography. The curcumin standard used was Sigma-Aldrich®, Merck, Germany, prepared in acetonitrile (J. T. Baker Chemical Company, USA) and the sample, in acetonitrile solution: ultrapure water (MilliQ®, Merck, Germany) 10:90 (v/v), filtered through a 0.45 µm x 13mm polyvinylidene fluoride (PVDF) membrane (Millipore®, Merck, Germany). The instrumental system used consisted of a Shimadzu Prominence LC-20A liquid chromatograph (Shimadzu Corporation, Kyoto, Japan), consisting of a CBM-20A communication module, CTO-20A column oven, SPD-M20A diode array detector at 425 nm, SIL-20AC HT automatic injector at 10 ºC, and pump with an LC-20AT quaternary phase mixer. The elution of the sample was performed using an isocratic mode with the mobile phases acetonitrile (JT Baker Chemical Company, USA): ultrapure water (MilliQ®, Merck, Germany) in the proportion 13:87 (v/v) for 15 minutes in a YMC ODS-AQ column (C18), 5 µm, 150 x 6.0 mm (Tedia Brazil, Brazil), with an injection volume of 20µL at a flow rate of 1.0 mL.min⁻¹. Data acquisition and processing were performed with the aid of LC solution software (Shimadzu Corporation, Kyoto, Japan).

**Animals and experimental protocol**

Figure 1 shows the descriptive flowchart of the experimental design. A total of 48 male Wistar rats of 90 days of age, from the Central Vivarium at the Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brazil, were randomly assigned to four groups: Control (CO), Turmeric (TU), Cinnamon (CI), and Okra (OK), composed of 12 rats each. Before the start of the experiment, the animals were kept for 7 days in our vivarium for acclimatization under the following conditions: four animals per cage, light and dark cycle of 12 hours (light on from 7 a.m.), fed with standard rodent chow diet (Nuvilab®, Nuvital, Brazil) and water ad libitum, and temperature controlled at 24 ± 1 ºC.

**Figure 1** - Descriptive flowchart of the experimental design.

**Source:** the authors.

After the acclimatization period, the animals were weighed and anesthetized intramuscularly with a combination of ketamine (Dopalen®, Sespo, Brazil; 100 mg/kg) and xylazine (Anasedan®, Sespo, Brazil; 20 mg/kg) to obtain blood samples, respecting a pre-harvesting fast of 15 hours. The animals were then divided into the four experimental groups, each receiving the respective treatment for 14 days (Fig. 1).

**IgA:** immunoglobulin A. CO: Control; TU: Turmeric; CI: Cinnamon; OK: Okra.
were then treated daily by gavage for 14 uninterrupted days. The CO group received 0.5 mL of phosphate buffered saline (PBS), pH 7.4, while the other groups (TU, CI, OK) received the samples (turmeric, cinnamon, and okra) prepared in 0.5 mL of PBS, corresponding to 50, 50, and 12.5 mg of each product, respectively.

At the end of the 14th day of treatment, after a pre-harvesting fast of 15 hours, the animals were weighed and anesthetized, as previously described, to obtain blood samples. The animals were then euthanized with a ketamine-xylazine overdose and dissected to obtain internal organs and tissues (peritoneal fat, adrenal gland, kidney, spleen, liver, and thymus). The organs and tissues were weighed, and the data presented as relative weight. The experimental protocol was approved by the Ethics Committee for Animal Research of the Universidade Estadual de Londrina (process number 8185.2014.18). All efforts were made to minimize the number of animals used and their suffering.

Biochemical analyses and total serum IgA measurement

To evaluate glucose levels (mg.dL⁻¹), the blood of the animals was collected by cardiac puncture in a test tube containing sodium fluoride (0.6 mg.mL⁻¹) and ethylenediaminetetraacetic acid (EDTA) (2 mg.mL⁻¹) before and after the treatment. The samples were then centrifuged at 1000 x g for 5 minutes. Plasma was recovered, aliquoted, and stored at -20 ºC until analysis.

The levels of triglycerides (mg.dL⁻¹), total cholesterol (mg.dL⁻¹), aspartate aminotransferase (AST) (U.L⁻¹), alanine aminotransferase (ALT) (U.L⁻¹), and humoral immunity were evaluated in serum after blood collection by cardiac puncture in a tube free of anticoagulants, before and after the treatment. Following coagulation, the samples were centrifuged at 1000 x g for 5 minutes. The serum was recovered and aliquoted and stored at -20 ºC until analysis.

Biochemical analyses were performed in a biochemical analyzer (Dimension® Clinical Chemistry System, Siemens, Germany) at the laboratory of the Veterinary Hospital of the Universidade Estadual de Londrina (HV-UEL) using available commercial kits.

Determination of IgA levels (mg.dL⁻¹) in serum was performed by indirect enzyme-linked immunosorbent assay (ELISA) using the Rat IgA ELISA Quantitation Set® quantification system (Bethyl Laboratories, Inc., USA), according to the manufacturer’s specifications, with modifications. Blocking was performed with PBS containing 5% skimmed milk (Molico®, Nestlé, Brazil) and dilutions of the standard curve, samples, and conjugated antibody were performed in PBS containing 1% skimmed milk.

Statistical analyses

The sample normality was verified using the Shapiro-Wilk test and the sample homogeneity by the Levene test. The one-way ANOVA test was performed to compare the variables between the groups in the pre- or post-intervention moment and to analyze the relative weight of the organs and tissues (differences between treatments at the same moment). In the intra-group comparisons, the paired “t” test was used (differences in the same group in the pre and post intervention moments). The statistical package SPSS® 23.0 (IBM, USA) was used, adopting a P value < 0.05.

Results

Table 1 presents the results of the characterization of the turmeric, okra, and cinnamon samples, regarding the physical-chemical composition, content of total phenolic compounds, and antioxidant capacity (DPPH and ABTS). Regarding the curcumin content of the Curcuma longa sample, a curcumin content of 5.1 ± 0.1% (dry basis) was observed.
The current study showed that the addition of turmeric or cinnamon (50 mg per day), as well as okra (12.5 mg per day) to the conventional diet of healthy Wistar rats did not change body mass and relative weight of adrenal gland, spleen, liver, peritoneal fat, kidney, and thymus (Table 3, p > 0.05). However, an increase in sera IgA levels was observed in the groups of animals treated with turmeric and cinnamon (Figure 2, p < 0.05).

**Table 1** - Centesimal composition of cinnamon, turmeric, and okra in terms of moisture, ash, protein, lipids and carbohydrates and total phenolic compounds and antioxidant capacity.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Turmeric</th>
<th>Okra</th>
<th>Cinnamon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humidity</td>
<td>10.0 ± 0.3</td>
<td>9.4 ± 0.3</td>
<td>10.9 ± 0.1</td>
</tr>
<tr>
<td>Ashes</td>
<td>5.1 ± 0.1</td>
<td>9.4 ± 0.1</td>
<td>4.5 ± 0.0</td>
</tr>
<tr>
<td>Proteins</td>
<td>7.9 ± 0.3</td>
<td>22.9 ± 1.9</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>Lipids</td>
<td>1.3 ± 0.6</td>
<td>1.1 ± 0.0</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>Carbohydrates*</td>
<td>75.7</td>
<td>57.2</td>
<td>76.2</td>
</tr>
<tr>
<td>Total phenolic compounds **</td>
<td>187.9 ± 1.2</td>
<td>120.5 ± 1.8</td>
<td>23.0 ± 0.8</td>
</tr>
<tr>
<td>DPPH***</td>
<td>4.0 ± 0.7</td>
<td>2.0 ± 0.5</td>
<td>47.1 ± 3.9</td>
</tr>
<tr>
<td>ABTS***</td>
<td>24.5 ± 6.8</td>
<td>14.7 ± 1.8</td>
<td>130.5 ± 7.6</td>
</tr>
</tbody>
</table>

**Caption**: values expressed in percentages and mean ± standard deviation; *values obtained by difference; **values expressed in mg GAE/g of sample; ***values expressed in TEAC (µM Trolox/g of sample). DPPH, radical 2,2-diphenyl-1-picrilhidrazil; ABTS, radical 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

**Source**: the authors.

**Table 2** - Body mass, blood biochemical parameters, and IgA blood levels pre- and post-treatment.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CO (n=12)</th>
<th>TU (n=12)</th>
<th>CI (n=12)</th>
<th>OK (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>348.6 ± 357.5</td>
<td>360.1 ± 364.5</td>
<td>380.3 ± 351.7</td>
<td>335.2 ± 347.7</td>
</tr>
<tr>
<td>Glu (mg.dL⁻¹)</td>
<td>164.1 ± 140.5</td>
<td>149.4 ± 159.8</td>
<td>159.4 ± 146.0</td>
<td>168.5 ± 147.1</td>
</tr>
<tr>
<td>Tgl (mg.dL⁻¹)</td>
<td>89.6 ± 108.5</td>
<td>79.3 ± 89.6</td>
<td>80.7 ± 90.7</td>
<td>76.5 ± 94.2</td>
</tr>
<tr>
<td>TC (mg.dL⁻¹)</td>
<td>68.8 ± 71.9</td>
<td>62.9 ± 68.7</td>
<td>71.8 ± 69.4</td>
<td>66.3 ± 69.0</td>
</tr>
<tr>
<td>AST (U.L⁻¹)</td>
<td>153.2 ± 136.0</td>
<td>160.0 ± 135.0</td>
<td>148.5 ± 181.4</td>
<td>158.3 ± 132.3</td>
</tr>
<tr>
<td>ALT (U.L⁻¹)</td>
<td>26.0 56.4</td>
<td>37.9 22.5</td>
<td>56.6 92.8</td>
<td>25.1 60.6</td>
</tr>
</tbody>
</table>

**Caption**: groups: Control (CO); Turmeric (TU); Cinnamon (CI); Okra (OK). Values expressed as mean ± standard deviation; Body mass: Mass, Glu: Glucose, Tgl: Triglycerides, TC: Total cholesterol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase; Pre: pre-treatment, Post: post-treatment. Values expressed as mean ± standard deviation; one-way ANOVA and paired “t” test (P < 0.05).

**Source**: the authors.
Effect of dietary plants on the production of immunoglobulins A in healthy Wistar rats

Discussion

IgA, as the principal antibody class in the secretions that bathe these mucosal surfaces, acts as an important first line of defense (23). It is also an important serum immunoglobulin, which mediates a variety of protective functions through interaction with specific receptors and immune mediators (19), neutralizing or blocking the activity of potential opportunistic intruders without starting an inflammatory response (24).

Turmeric is a dietary plant with an immunomodulatory effect, and in this sense, the literature has reported that it increases the intestinal immune function in high-fat fed animals by increasing IgA production or suppressing IgA degradation (25). This increase in IgA production was observed in animals treated with turmeric for 14 days at doses of 5 and 10 mg/kg; the higher the dose, the greater the production of IgA (17). It is important to note that the animals treated with turmeric presented greater production of IgA and protection against

Table 3 - Relative weight of the organs and tissues post-treatments.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CO (n=12)</th>
<th>TU (n=12)</th>
<th>CI (n=12)</th>
<th>OK (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal gland (g)</td>
<td>0.016 ± 0.013</td>
<td>0.018 ± 0.008</td>
<td>0.015 ± 0.013</td>
<td>0.018 ± 0.012</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.145 ± 0.055</td>
<td>0.152 ± 0.018</td>
<td>0.139 ± 0.050</td>
<td>0.142 ± 0.053</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>2.912 ± 0.998</td>
<td>3.072 ± 0.346</td>
<td>2.692 ± 0.940</td>
<td>2.637 ± 0.898</td>
</tr>
<tr>
<td>Peritoneal fat (g)</td>
<td>0.825 ± 0.496</td>
<td>0.848 ± 0.326</td>
<td>0.635 ± 0.263</td>
<td>0.735 ± 0.341</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>1.184 ± 0.407</td>
<td>1.180 ± 0.208</td>
<td>1.114 ± 0.408</td>
<td>1.059 ± 0.394</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td>0.037 ± 0.023</td>
<td>0.036 ± 0.016</td>
<td>0.036 ± 0.023</td>
<td>0.031 ± 0.024</td>
</tr>
</tbody>
</table>

**Caption**: groups: Control (CO); Turmeric (TU); Cinnamon (CI); Okra (OK). Values expressed as mean ± standard deviation; one-way ANOVA ($P < 0.05$).

**Source**: the authors.

Figure 2 - Evaluation of IgA concentration in rats with different treatments. Values expressed as mean ± standard deviation. IgA: immunoglobulin A. Pre: pre-treatment; Post: post-treatment; * indicates difference in the intragroup moments. Values expressed as mean ± standard deviation; one-way ANOVA and paired “t” test ($P < 0.05$).

**Source**: the authors.
Salmonella typhimurium-induced infection compared to the control.(26) Although the mechanisms of action of turmeric in the production of IgA have not yet been defined, there is evidence that this may be related to curcuminoid pigments.(15) These pigments have been shown to exhibit anti-inflammatory activity through the suppression of numerous cell signalling pathways including NF-κB, STAT3, Nrf2, and COX-2.(27) Clinically, they have been shown to be effective against various chronic diseases, including various types of cancers, diabetes, obesity, and cardiovascular, pulmonary, neurological, and autoimmune diseases.(27)

This is the first study to find an effect of cinnamon on the production of IgA antibodies. Studies suggest that cinnamon has anti-inflammatory properties. Animals treated with cinnamon for 20 days presented a reduction in pro-inflammatory cytokines (IL-1β, IFN-γ, and TNF-α) and a significant increase in the production of anti-inflammatory cytokines (IL-10).(28) Thus, as the importance of IL-10 in the regulation of B cell class switching has been reported in the production of IgA,(29) this immunomodulatory effect could explain the fact that cinnamon elicited high IgA levels in the animals in this study.

Conclusion

The current work shows that rats fed a conventional diet with added turmeric, cinnamon, and okra did not present significant changes in body mass, relative weight of organs and tissues, and biochemical parameters. Furthermore, turmeric and cinnamon raised IgA blood levels, which may indicate immunomodulation. Although the mechanisms of action of turmeric and cinnamon in the production of IgA have not yet been defined, our study supports the idea that dietary supplementation with these plants may improve humoral immunity.

Conflicts of interest

The authors declare no conflict of interest.

References

Effect of dietary plants on the production of immunoglobulins A in healthy Wistar rats


27 Kunnumakkara AB, Bordoloi D, Padmavathi G, Monisha J, Roy NK, Prasad S, et al. Curcumin, the golden nutraceutical: multitargeting
