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Corrective effects of acerola (*Malpighia emarginata* DC.) juice intake on biochemical and genotoxical parameters in mice fed on a high-fat diet

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ABSTRACT

Acerola contains high levels of vitamin C and rutin and shows the corresponding antioxidant properties. Oxidative stress on the other hand is an important factor in the development of obesity. In this study, we investigated the biochemical and antigenotoxic effects of acerola juice in different stages of maturity (unripe, ripe and industrial) and its main pharmacologically active components vitamin C and rutin, when given as food supplements to obese mice. Initial HPLC analyses confirmed that all types of acerola juice contained high levels of vitamin C and rutin. DPPH tests quantified the antioxidant properties of these juices and revealed higher antioxidant potentials compared to pure vitamin C and rutin. In an animal test series, groups of male mice were fed on a standard (STA) or a cafeteria (CAF) diet for 13 weeks. The latter consisted of a variety of supermarket products, rich in sugar and fat. This CAF diet increased the feed efficiency, but also induced glucose intolerance and DNA damage, which was established by comet assays and micronucleus tests. Subsequently, CAF mice were given additional diet supplements (acerola juice, vitamin C or rutin) for one month and the effects on bone marrow, peripheral blood, liver, kidney, and brain were examined. The results indicated that food supplementation with ripe or industrial acerola juice led to a partial reversal of the diet-induced DNA damage in the blood, kidney, liver and bone marrow. For unripe acerola juice food supplementation, beneficial effects were observed in blood, kidney and bone marrow. Food supplementation with vitamin C led to decreased DNA damage in kidney and liver, whereas rutin supplementation led to decreased DNA damage in all tissue samples observed. These results suggest that acerola juice helps to reduce oxidative stress and may decrease genotoxicity under obesogenic conditions.

1. Introduction

Obesity is widely recognized as one of the largest and fastest growing problems for the general public health [1]. Obesity severely affects not only individuals' health and quality of life, but also strains healthcare systems. In order to understand the abnormal homeostasis, which is responsible for most cases of human obesity, rodent models are frequently used [2]. Previous studies have shown that rodents fed on CAF diets, i.e. diets rich in sugar, salt and fat, are more likely to develop obesity and metabolic syndrome, which ultimately results in a shorter lifespan [2,3]

Risk factors for obesity include increased consumption of food, which is both dense in energy and poor in nutrients (CAF diets) [4]. The fact that this type of food is usually highly palatable moreover facilitates a higher calorie intake [3]. Diets with a proportionally higher contribution of energy-dense food also increase the risk of an insufficient intake of calcium (Ca), potassium (K), iron (Fe), zinc (Zn), and fibers. On the other hand, the probability of consuming

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excessive amounts of added sugar, fat, and sodium (Na) is increased [4].

Oxidative stress and inflammation are closely correlated in all types of tissue, which are affected by obesity. Oxidative stress causes an increased production of inflammatory cytokines, which promote the production of free radicals. The increased production of free radicals results in oxidative damage to macromolecules, which can be aggravated by an impaired ability of the cells to quench these radicals and repair damaged molecules [5]. Some aspects of the connection between obesity, oxidative stress and DNA damage has been described in previous studies [6,7].

As obesity is worsened by oxidative stress, obese individuals may benefit from food supplementation with antioxidants [8]. Appropriate diet modifications may prevent, decelerate or stop the development of obesity. Regardless of antioxidant properties, fruit and vegetables are an extremely important staple of the human diet, as they contain substantial quantities of fibers, vitamins and minerals, which have been linked to the prevention of cardiovascular diseases [9]. They also contain phytochemicals, which function as antioxidants, phytoestrogens, and anti-inflammatory agents [10].

The Barbados or acerola cherry (*Malpighia emarginata* DC.) is native to South and Central America, and cultivated in tropical or subtropical climate zones [11,12]. Acerola contains high levels of vitamin C, and important flavonoids such as rutin [11,13–15]. Therefore, acerola is usually consumed prophylactically or in a supporting capacity to other treatment strategies. Vitamin C is a potent antioxidant, mainly due to its ability to efficiently quench free radicals, which are produced during a variety of biological processes [16,17]. Rutin is a physiological antioxidant, able to effectively quench free radicals and prevent lipoperoxidation by chelating metal ions [10].

Previous in vitro studies suggested beneficial antigenotoxic and antioxidant properties for acerola juice. These were derived from the ability of acerola juice to quench the free radical di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH). The DPPH test is commonly used in order to quantify the antioxidant properties of substances in food sources (e.g. vitamins or flavonoids) [14,15].

In this study, we investigated the antioxidant potential of acerola juice in different stages of maturity (unripe, ripe and industrial). We also examined the biochemical and antigenotoxic effects of food supplementation with these juices in order to understand the relation between oxidative stress and DNA damage in obesity. To that purpose, a CAF diet was used to induce obesity and metabolic syndrome in mice. The resulting metabolic disorder was characterized by oral glucose tolerance tests. In addition, we investigated the biochemical and antigenotoxic effects of food supplementation with isolated rutin and vitamin C, with the aim to elucidate further information about their protective mode of operation.

2. Materials and methods

2.1. Chemical reagents

Ripe and unripe acerola cherries (*M. emarginata* DC.) were purchased from Nutrilite Farm (Ceará, Brazil). Industrial acerola juice was obtained from Da Fruta® (Pernambuco, Brazil). Cherries were received frozen from the manufacturer and stored at −20 °C. Immediately prior to the experiments, cherries were thawed and processed in a juice extractor. Juices were administered to the animals by gavage. Industrial acerola juice was stored at −4 °C. L-ascorbic acid (Vitamin C; CAS Number 50-81-7), rutin hydrate (CAS Number 207671-50-9), ethidium bromide staining solution (CAS Number 1239-45-8), and Giemsa staining solution (CAS Number 1.09204.1002) were purchased from Sigma–Aldrich (Porto Alegre, Brazil). In order to obtain the desired doses, L-ascorbic acid and rutin hydrate were dissolved in distilled water prior to each experiment. All experiments were carried out in minimal indirect light.

2.2. Animals

For this study, 42 healthy, male Swiss albino mice (average body weight: 25 ± 0.5 g; age: 5–6 weeks) were obtained from the Animal Center of the University of Southern Santa Catarina (UNESC, Brazil). All experimental procedures were approved by the local ethics committee for animal use (CEUA – UNESC; Register No. 130/2011). All procedures involving animals and their care were also carried out in accordance with national and international laws and guidelines for the use of animals in biomedical research. The animals were randomized by weight and housed in polycarbonate cages with steel wire tops (6 animals/cage). Cages were exposed to alternating light/dark cycles of 12 h and kept at standard room temperature (22 ± 2 °C) and humidity (55 ± 10%). Meticulous efforts were made to reduce external sources of stress, pain and discomfort for the animals to a minimum. Only the absolutely necessary number of animals was used, in order to produce reliable scientific data.

2.3. Experimental design

Prior to the experiments, all the animals were given one week to acclimatize to their new environment. The mice were subsequently divided into a control group of six animals, fed on a standard diet (STA) and a cafeteria diet (CAF) group of 36 animals. Both groups were kept on their respective diet for 13 weeks. After this period, the CAF group was divided into six CAF subgroups (6 animals/group), which were administered 0.1 mL/10 g/d of the following food supplements: (1) water, (2) unripe acerola juice, (3) ripe acerola juice, and (4) industrial acerola juice. Subgroup (5) was administered 1 mg/kg/d of vitamin C, whereas subgroup (6) was given 200 mg/kg/d of rutin. Administered doses of vitamin C and rutin were chosen according to Franke et al. [17] and La Casa et al. [18], respectively.

The food supplements vitamin C and rutin were included in these study as they occur in high concentrations in acerola juices (vide infra). Moreover, rutin is the glycoside of the flavonol quercetin and the disaccharide rutinose (α-L-rhamnopyranosyl-(1 → 6))-β-D-glucopyranose) and is metabolized to the glycone quercetin, which is responsible for an in vivo antioxidant activity [19].

The food intake was recorded on a daily basis, and all animals were weighed weekly. After 30 days of treatment with the respective food supplements, the animals were killed. At 9 am of the 30th day, mice were given the last dose of their respective food supplement and the base diet supply was renewed. From midnight of the same day onwards, access to food was denied and after a fasting period (8 h), animals were decapitated. Afterwards, blood and tissue samples were collected for DNA analysis. The entire experiment was conducted during a period of 17 weeks.

2.4. Experimental diets

The palatable high-calorie CAF diet was chosen, because of its resemblance to modern patterns of human food consumption and due to its track record in inducing obesity in lean animals [21]. The CAF diet used in this study was adapted from a diet previously

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