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Chemopreventive activity of apple extract following medium-term oral carcinogenesis assay induced by 4-nitroquinoline-1-oxide

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ABSTRACT

Objective: The aim of this study was to evaluate the chemopreventive activity of an apple extract following medium-term oral carcinogenesis assay induced by 4-nitroquinoline-1-oxide (4NQO).

Methods: A total of 30 male Wistar rats were distributed into five groups as follows ($n = 6$ per group): Group 1, negative control group (non-treated group); Group 2, received 4NQO during 8 weeks in drinking water and treated with apple extract at 1% by gavage between the first and fourth weeks daily (initiation phase); Group 3, received 4NQO for 8 weeks in drinking water and treated with apple extract by gavage at 1% between the fifth and eighth weeks daily (promotion phase); Group 4, received apple extract at 1% by gavage for 8 consecutive weeks only; and Group 5, received 4NQO for 8 weeks in drinking water daily.

Results: Histopathological analysis revealed decreased hyperplasic lesions in Group 2 when compared with Group 5. Likewise, decreased dysplastic lesions in Group 3 were observed when compared with Group 5. In Groups 2 and 3, decreased COX-2 and TNF-alpha gene expressions were observed when compared with Group 5. Cytochrome c and caspase 3 levels increased in Groups 2 and 3 when compared with Group 5.

Conclusion: In conclusion, our results demonstrate that apple extract suppresses rat tongue carcinogenesis as a result of anti-inflammatory activity and apoptosis through the intrinsic mitochondrial pathway.

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1. Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer and is the sixth most common human malignancy, with a 5-year survival rate of approximately 65%.[1] The use of 4-nitroquinoline-1-oxide (4NQO) in rat tongue is a reliable procedure for inducing OSCC because it simulates rat tongue carcinogenesis in a similar way in which carcinogenesis develops in humans.[2,3] 4NQO is a water-soluble quinoline derivative and is known to form DNA adducts.[4] In bacteria, 4NQO can induce changes between base pairs (GC–AT) and cause deletion mutations.[5] In rats, 4NQO is able to induce oxidative DNA damage, chromosomal breakage as well as oxidative DNA lesions.[6] 4NQO is considered a potent carcinogen in many organisms due to its ability to specifically induce squamous cell carcinoma in tongue when dissolved in drinking water.[7]

Accumulating evidence suggests that inflammation contributes to the development of cancer and also that cancer seems to directly promote the generation of an inflammatory microenvironment.[8] However, it is not clear if inflammatory conditions increase the local cancer risk or if genetic alterations such as oncogenes cause inflammation and neoplasia.[8] Nevertheless, some cytokines are closely involved in all inflammatory condition reactions.[9] Overproduction of proinflammatory, proangiogenic cytokines by oral squamous cancer cells has been reported and their role in tumour progression and angiogenesis has been established.[10,11]

Apoptosis, also known as programmed cell death, is characterized by typical cellular morphology and biochemical features such as cell shrinkage, cytoplasm vacuolization, chromatin condensation, DNA fragmentation, and finally cellular breakdown into apoptotic body.[12] It has been assumed that mitochondria play essential roles in triggering apoptosis through the mitochondrial permeability transition.[13] The mitochondrial permeability transition leads to the loss of mitochondrial membrane potential and produces translocation of proapoptotic Bax to mitochondria and cytochrome c (Cyt C) from mitochondria to cytosol resulting in the activation of caspases.[8] Recent studies have proposed that cellular reactive oxygen species (ROS) induced by oxidative stress may mediate mitochondria-initiated apoptosis.[14] Herein, some chemopreventive agents can lead to the activation of apoptosis through decreased cellular ROS production, as a result of mitochondrial apoptotic mediators.[15]

The intelligent use of food byproducts to obtain new products and ingredients has been extensively pursued in recent years.[16] Innovative foodstuff have been patented using polyphenols as promoters of health benefits, as recently reviewed by Gollücke and Ribeiro.[17] Apple extract is a new product obtained by washing the residues resulting from apple juice production and by recovering the polyphenols still present in apple seeds and peel after juice extraction. Some authors have demonstrated that apple exhibits efficient antioxidant properties owing to the presence of its phytoconstituents that are able to exert anti-inflammatory, antiviral, and antimicrobial properties.[18]

As a result, and because of the lack of scientific evidence, the aim of this study was to evaluate the anti-inflammatory potential of apple extract following a medium-term oral carcinogenesis assay induced by 4NQO in rats. Certainly, such data will contribute to a better understanding of the outcomes of nutraceutical compounds such as apple polyphenols against oral cancer progression as a result of their anti-inflammatory action.

2. Materials and methods

2.1. *Animals and experimental design*

All experimental protocols involving animals conformed to procedures described in the Guiding Principles for the Use of Laboratory Animals. The study was approved by the Animal Committee of Federal University of São Paulo, UNIFESP (number 0232/12). A total of 30 Wistar rats (8 weeks old) weighing approximately 250 g were obtained from Centro de Desenvolvimento de Modelos Experimentais (CEDEME), Federal University of São Paulo, SP, Brazil. They were maintained under controlled conditions of temperature (24 ± 2 °C), light–dark periods of 12 h, and with free access to water and commercial diet (Nuvital®, PR, Brazil).

A total of 30 male Wistar rats were distributed in five groups, as follows ($n = 6$ per group): Group 1, negative control group (non-treated group); Group 2, received 4NQO (Sigma–Aldrich®, St. Louis, MO, USA) at a dose of 20 ppm during 8 weeks in drinking water and treated with apple extract at 1% dose orally by gavage between the first and fourth weeks daily; Group 3, received 4NQO (Sigma–Aldrich®, St. Louis, MO, USA) at a dose of 20 ppm for 8 weeks in drinking water and treated with apple extract at 1% dose orally by gavage between the fifth and eighth weeks daily; Group 4, received apple extract at a dose of 1% by gavage for 8 consecutive weeks only; and Group 5, received 4NQO for 8 weeks at a dose of 20 ppm in drinking water daily. The experimental periods were established to evaluate the outcomes of apple extract on the initiation phase (first–fourth weeks) and the promotion phase (fifth–eighth weeks) following carcinogen exposure as described elsewhere.[19] The rats were sacrificed by administering 0.4% sodium pentobarbital (1 mL/kg, i.p.).

2.2. *Quantification of total phenols and determination of radical scavenging activity of apple extract*

The extract was obtained from Golden Sucos (Farroupilha-RS, Brazil) at 65° Brix (65% soluble solids). For quantification of total phenols, the Folin–Ciocalteu assay was used.[20] The method applies gallic acid (Sigma–Aldrich®, St. Louis, MO, USA) for the standard curve and the results are expressed in milligramme gallic acid equivalents (GAE)/kg. The readings (in triplicates) were taken at 740 nm using a Genesis 2 spectrometer (Thermo Scientific, California, USA). In order to assess the antioxidant activity in vitro, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma–Aldrich®, Steinheim, BW, Germany) assay was used based on the methods of Brand-Williams et al.[20] The absorbance was measured with a Beckman spectrometer (Beckman Coulter, California, USA) at 517 nm before the addition of samples and after 30 min; the difference was plotted on a vitamin C (ascorbic acid) (Merck®, Whitehouse

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