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In vivo antioxidant effect of aqueous and etheric coriander (*Coriandrum sativum* L.) extracts

Research has shown that aqueous and etheric coriander extracts, composed of phenolics and carotenoids, exhibit a considerable antioxidant action. Considering the importance of natural antioxidants in the reduction of free radicals and oxidised compounds, coriander extracts obtained through sequential extraction, were administered by gavage to Wistar rats during 30 and 60 d consecutively. At the end of each experimental period, the animals were sacrificed to obtain the blood and the liver. The effect of extracts on plasma and liver lipid peroxidation was assessed by evaluation of thiobarbituric reactive substances. Initially, the aqueous extract demonstrated a superior antioxidant activity in plasma but this was replaced by the etheric extract by the end of the experiment. Of the 2 extracts, in the etheric exerted a superior antioxidant activity in both liver and plasma. The effectiveness of the extracts appears to be related to the biological material that is used (liver or plasma), furthermore to the duration of administration and the type of extract.

Keywords: Coriander extracts, antioxidants, biologic effect, phenolic compounds, carotenoids.

1 Introduction

Epidemiological and experimental evidence has demonstrated that free radicals, resulting from oxidation reactions, are associated with pathological processes [1, 2]. This finding has motivated the search for antioxidant compounds found naturally in foodstuffs, particularly phenolics and carotenoids, with the aim of preventing or minimising oxidative damage to the organism [3]. In this context, aqueous and etheric coriander extracts (*Coriandrum sativum* L.), composed of a variety of phenolic and carotenoid compounds respectively, were found to exhibit considerable inhibition of lipid oxidation [4].

The maintenance of organic levels of dietary antioxidants is, according to *Vannucchi* et al [5], an important factor contributing to the reduction of free radical damage. For this reason several authors have studied the antioxidant potential of many vegetables, their extracts or component compounds [3, 6-12]. Despite the observations of *Frankel* et al. [13] and *Mendsen* and *Bertelsen* [14], who describe that the data obtained *in vitro* depend highly on the experimental conditions used, most of the studies on this field have been carried out only *in vitro*. Also *Cintra* and *Mancini Filho* [15] have observed differing antioxidant activities in oregano and rosemary depending on the *in vitro* systems used. In light of these facts and considering the importance of *in vivo* studies, the present study was designed to investigate the antioxidant effect of coriander extracts on rat plasma and liver.

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2 Material and methods

Etheric and aqueous coriander extracts were obtained through a sequential extraction process. The powdered coriander (80 mesh) was extracted with ethyl ether, agitating for 60 min at room temperature (25 °C±2 °C), followed by centrifugation at 3,000 *g* for 10 min. The residue was added to ethanol and subsequently to distilled water, under conditions described above. The etheric extract was evaporated under reduced pressure at 35 °C, thereafter drops of Tween 40 (*Merck*, Darmstadt, Germany) and distilled water were added and the mixture vigorously agitated to obtain an emulsion. This emulsion and the aqueous extract, after an application of nitrogen, were stored in amber glasses and maintained frozen at –18 °C throughout the experiment, whilst the ethanolic extract was discarded.

2.1 *In vivo* antioxidant activity evaluation

80 male, 21 d old Wistar albino rats from the Breeding Laboratory of the Nutrition Department – UFPE, weighing 50±2 g, were randomly distributed into 2 groups of 40 animals according to the study duration (30 or 60 d). Each group was subdivided into 4 sub-groups of 10 animals: control (C), standard (S), aqueous extract (A), and etheric extract (E). An emulsion with the following content was

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administered daily to these respective sub-groups during 30 and 60 d: 1 ml of 0.1 M phosphate buffer solution pH 7.4, 1 ml of BHT solution (100 ppm), 1 ml of aqueous coriander extract containing 0.4 mg of total phenolic and 1 ml of etheric coriander extract with 15 µg of total carotenoids. The animals were maintained in individual metabolic cages, at 25 °C ± 2 °C and on a 12-h light/dark cycle. Commercial feed (labina®, *Ralston Purina do Brasil,* São Paulo, Brazil) and water were provided *ad libitum*.

At the end of the experimental period (30 or 60 d), blood samples were collected by cardiac puncture and the liver was excised from animals anaesthetised with ethyl ether. The blood was transferred to test tubes containing 200 µl of 5% EDTA, and the plasma was obtained after centrifugation at 3,000 *g* for 10 min. After macroscopic inspection and weighing, a sample of liver tissue was homogenised immediately in 0.1 M phosphate buffer pH 7.4 in the proportion 1:10 (p/v). The homogenate was centrifuged at 1,400 *g* for 10 min and the protein content was determined by the spectrophotometric method of *Lowry* et al. [16], using bovine albumin as the reference standard. Aliquots of this homogenate and of the plasma were taken to determine the concentration of thiobarbituric reactive substances (TBARs), using as a standard 1,1,3,3-tetramethoxypropane (TMP), according to the method described by *Winterbourn, Gutteride* and *Halliwell* [17]. In addition, the body weight gain of each animal was assessed at the end of the experiment. The Ethics Commission for Animal Experimentation (CEEA) of the Federal University of Pernambuco approved the procedure, in accordance with the International Regulations of the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.2 Statistical analysis

The obtained data were submitted to variance analysis (F-test) and the *Tukey* test, at the 5% significance level, using the statistical programme "*Minitab for Windows*" (*Minitab* Inc., State College, PA, USA).

3 Results and discussion

Neither deaths nor behavioural abnormalities or alterations in the animals were registered during the experiment. There were no differences in body weight gain and relative liver weight ($p > 0.05$) between the different sub-groups (Tab. 1). These observations indicate that coriander extract at the doses used is safe, however this should be confirmed with toxicological studies.

Justesen and Knuthsen [18] reported the content of flavonoids in coriander to be 5 mg of quercetin in 100 g of fresh weight. On a previous study, caffeic acid and β-carotene were identified as major polyphenol and carotenoid of the coriander aqueous and etheric extracts, respectively [4]. *Ramos* and *Rodriguez-Amaya* [19] also detected the presence of β-carotene and lutein in fresh coriander leaves.

In the context of *Young* and *Lowe*'s premise [20] that the low solubility of the carotenoids in an aqueous solution limits its antioxidant action in the hydrophobic regions in biological systems, it would be reasonable to presuppose that the etheric extract may be more effective in the liver homogenate and the aqueous extract in the plasma due to the predominance of phenolic compounds in the last one. The concentration of TBARs in the liver of animals from sub-groups A and E, support this hypothesis by demonstrating significant superiority of the etheric extract compared to the aqueous after 30 d. The antioxidant effect of the etheric extract was also better than the standard solution (BHT), including the day 60, although not significantly (Tab. 2). With respect to the plasma, the slight superiority of the aqueous extract over the etheric at the 30-day period was supplanted, significantly, at the 60-day period.

Tab. 1. Body weight gain and relative liver weight of rats treated during 30 and 60 d with aqueous and etheric coriander extracts. C: Control (phosphate buffer pH 7.4); S: standard (BHT 100 ppm); A: aqueous extract; E: etheric extract (emulsion)†.

Sub-groups	30 d		60 d	
	Body weight gain [g]	Relative liver weight [g/100g]‡	Body weight gain [g]	Relative liver weight [g/100g]‡
C	221 ± 37	4.0 ± 0.2	270 ± 47	3.8 ± 0.2
S	234 ± 32	4.1 ± 0.2	275 ± 28	3.7 ± 0.2
A	191 ± 29	4.2 ± 0.3	270 ± 19	4.0 ± 0.3
E	229 ± 19	4.4 ± 0.2	279 ± 28	3.6 ± 0.2

† Mean values in a column did not differ statistically (*Tukey* test at the 5% significance level); each value is expressed as mean ± SD of 10 animals.
‡ g/100g of rat body weight.

Tab. 2. Effect of aqueous and etheric coriander extracts on plasma and liver levels of TBARs. C: Control (phosphate buffer pH 7.4); S: standard (BHT 100 ppm); A: aqueous extract; E: etheric extract (emulsion)†.

Sub-groups	TBARs levels			
	After 30 d of treatment‡		After 60 d of treatment‡	
	Liver [mmol/mg protein]	Plasma [mmol/ml]	Liver [mmol/mg protein]	Plasma [mmol/ml]
C	1.9 ± 0.4^{a}	0.45 ± 0.04^{a}	2.25 ± 0.33^{a}	0.92 ± 0.11^{a}
S	1.0 ± 0.2^{bc}	0.15 ± 0.03^{b}	0.89 ± 0.29^{b}	0.306 ± 0.05^{bc}
A	1.3 ± 0.5^{b}	0.25 ± 0.03^{c}	1.06 ± 0.21^{b}	0.356 ± 0.03^{b}
E	0.9 ± 0.2^{c}	0.25 ± 0.01^{c}	0.86 ± 0.12^{b}	0.243 ± 0.06^{c}

† Each value is expressed as mean ± SD of 10 animals. Mean values with different letters in a column are significantly different (*Tukey* test at the 5% significance level).
‡ Control, standard, aqueous and etheric coriander extracts were administered daily by gavage to these respective sub-groups during 30 and 60 d.

This finding may have been affected by other antioxidants found naturally in the organism, as observed by *Palozza* et al [21]. This author administered 14 µg/g of body mass canthaxanthin for 15 d to animals by a technique identical to that used in this study, and observed, that although the liver TBARs concentration remained unaltered, there was a reduction in the glutathion peroxidase activity and an increase in the catalase and superoxide dismutase activity. *Jain, Agarwal* and *Rao* [22] observed after adding 10 ppm lycopene in the oleoresin form to the diet of rats an increase of 8% in thiol concentration in addition to a reduction in plasma TBARs level of around 14%.

The studies of *Banerjee* et al [23, 24] using aqueous garlic extract, predominantly composed of phenolic compounds, during 30 d, support the results presented here. In addition to reductions in the TBARs levels, in the cardiac, hepatic and kidney tissues, an increase in the activity of endogenous antioxidant enzymes was observed in particular that of superoxide dismutase. According to *Nardini* et al [25], dietary supplementation of caffeic acid (0.8%) in rats for 6 weeks resulted in a statistically significant increase of α-tocopherol both in plasma and lipoprotein with a direct contribution to the antioxidant defence system. *Zhang* et al [26] supported this finding by adding 2% hawthorn fruit powder (*Crataegus pinnatifida*), composed of epicatechin, chlorogenic acid, isoquercitrin, protocatechuic acid, rutin and quercetin to the diet of rats. These findings demonstrate the potential of phenolic and carotenoid compounds to increase the efficiency of the organic defence system, either through a direct action or by reducing the depletion of endogenous antioxidants.

As the animals aged there was a progressive increase in the plasma TBARs level in the control, the standard and the A sub-groups, differently to that observed in the liver homogenate of all except the control sub-group. Given that a progressive process of oxidation occurs during ageing, this result shows that the etheric coriander extract is capable of reducing this tendency in the animal liver. As can be seen in Fig. 1, the efficacy of the extracts increased with the duration of their administration. The aqueous extract demonstrated a superior efficacy in plasma, whereas the etheric extract was more effective in hepatic tissue. After 60 d, peroxidation was also in plasma inhibited to a higher percentage by the etheric extract than by the aqueous extract.

Carotenoids are regarded as effective antioxidants. Their efficacy, either in biological systems or in food, seems to be related to the ability to act as singlet oxygen quenchers, the highly reactive form of oxygen, and/or interact with free radicals [27]. There is no evidence to support the hypothesis that these substances may act as prooxidants within a biological system. However, on the basis of their performance *in vitro*, some factors may

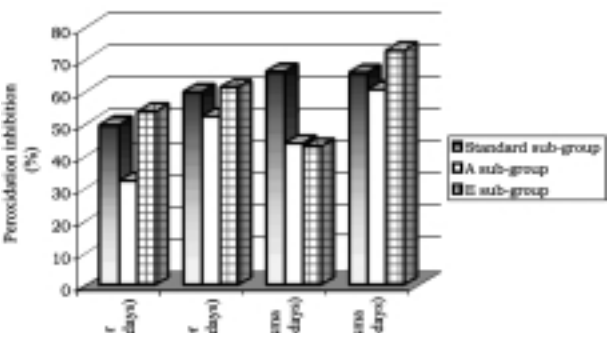

Fig. 1. Peroxidation percent inhibition of lipids in the liver and plasma of rats treated during 30 and 60 d with aqueous and etheric coriander extracts, calculated from control (100% of oxidation).

serve to reduce the antioxidant effectiveness of carotenoids *in vivo* [20].

Even though little is known of the bioavailability and metabolism of phenolic compounds, it is possible to infer from the results obtained in this study that bioactive compounds from coriander extracts can be absorbed from the gastrointestinal tract. According to *Scalbert* et al [28], caffeic acid is easily absorbed through the gut barrier, whereas large molecular weight polyphenols are very poorly absorbed. The absorption of this phenolic acid was higher (95%) than that of chlorogenic acid (33%) in iliostomised human subjects [29]. *Hempel* et al [30] administered salvia extract (0.71 mg of flavonoids/kg of body mass) by gavage to adult rats. They observed a maximum plasma antioxidant capacity 1 h after administration of the extract, returning gradually to the normal value after about 12 h and showing a slight increase 24 h after administration. They explained the late rise in activity as a possible action of phenolic compounds that entered the plasma after passing the enterohepatic circulation. In a recent study with volunteers that had ingested 400 ml of mixed fruit juice containing 2470 mg/l of total phenolic (gallic acid equivalents) and 415 mg/l of anthocyanins, *Netzel* et al [11] observed a significant rise in plasma antioxidant capacity 2 h after ingestion. This confirmed the antioxidant action of these compounds and their absorption by the organism.

Chemical tests, performed in the initial stages of this study did not reveal important differences in antioxidant action between etheric and aqueous extract [4]. This differs from the results obtained *in vivo* and therefore demonstrates the importance of this type of evaluation to verify their action in reducing oxidative stress in the organism. The coriander aqueous and etheric extracts at the dose used (3.5 ml/kg body weight), corresponding to 1.5 mg and 50 μg/kg body weight of the total phenolics and carotenoids, respectively exerted good protective effect on rats. However, the effect of these extracts on the suppression of lipid peroxidation in adult human needs to be examined and its dose determined.

4 Conclusion

The results demonstrate that bioactive compounds present in aqueous and etheric coriander extracts exert an *in vivo* protective effect against lipid peroxidation, reversing this alteration during the ageing process. The effectiveness of these extracts improved with prolonged and continued duration of use. The etheric extract, however, was more effective in reducing oxidation.

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