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Life Sciences 67 (2000) 3023–3029

Life Sciences

High levels of melatonin in the seeds of edible plants Possible function in germ tissue protection

Lucien C. Manchester[b], Dun-Xian Tan[a], Russel J. Reiter[a,*], Won Park[b], Kanishka Monis[b], Wenbo Qi[a]

[a]Department of Cellular and Structural Biology, Mail Code 7762, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78284, USA

[b]Department of Biological Sciences, St. Mary's University, San Antonio, TX 78228-8511, USA

Received 22 March 2000; accepted 19 May 2000

Abstract

The seeds of plants represent the anlage of the next generation and are vital to their existence. Melatonin has been identified in the leaves and flowers of plants but not in seeds. In this study, we examined the seeds of 15 edible plants for the presence of melatonin which was extracted using cold ethanol. Melatonin was initially identified by radioimmunoassay and subsequently quantified and confirmed using high performance liquid chromatography. The physiological concentrations of melatonin in the 15 seeds studied ranged from 2 to 200 ng/g dry weight. The highest concentrations of melatonin were observed in white and black mustard seeds. This level of melatonin is much higher than the known physiological concentrations in the blood of many vertebrates. Since the seed, particularly its germ tissue, is highly vulnerable to oxidative stress and damage, we surmise that melatonin, a free radical scavenger, might be present as an important component of its antioxidant defense system. Thus, melatonin in seeds may be essential in protecting germ and reproductive tissues of plants from oxidative damage due to ultraviolet light, drought, extremes in temperature, and environmental chemical pollutants.

Keywords: Seeds; Plants; Melatonin; Free-radical; Germ tissue; Antioxidant

Introduction

Following the isolation and identification of melatonin from bovine pineals [1], much of the research on melatonin has centered around its presence in vertebrates. Historically, melatonin, an indole derivative of tryptophan, was considered unique to the pineal gland but more recently, however, melatonin has been identified in variety of other tissues [2]. Also, melato-

* Corresponding author. Fax: 210-567-6948.
E-mail address: Reiter@uthscsa.edu (R.J. Reiter)

0024-3205/00/$ – see front matter © 2000 Elsevier Science Inc. All rights reserved.
PII: S0024-3205(00)00896-1

nin has been found in every kingdom including Monera (*Rhodospirillum rubrum*) [3], Protista (*Gonyaulax polyedra*) [4], Fungi (*Saccharomyces cerevisiae*) [5], and Plantae [6,7]. Interest in the presence of melatonin in plants was hightened with the discovery of large amounts of melatonin in two so-called medicinal herbs, feverfew and St. John's wort [8].

Melatonin has been identified using high performance liquid chromatography (HPLC) in a number of edible plants, including fruits, tomato, rice, oranges, apples, banana, and cabbage [6]. Coincidentally, Dubbels and coworkers [7] also found melatonin in tomato, beet, potato, and bananas. Since these initial discoveries, scientists have concerned themselves with the extent of the distribution of melatonin in plants.

In most animals, melatonin acts as a zeitgeber coordinating responses to diurnal and photoperiodic signals in the environment. Pineal melatonin exhibits a daily rhythm with highest levels seen in the scotophase and lowest levels seen during the day or photophase [9,10]. Kolar and colleagues [11] reported that in the plant, *Chenopodium rubrum*, a diurnal rhythm of melatonin also existed with highest levels being present during the night to early morning and lowest levels during the day. This observation contrasts with findings in aloe vera and St. John's wort in which high levels of melatonin were found uniformly over the day and night (Manchester et al., unpublished).

The extremely high levels (μg/g dry plant tissue) of melatonin in feverfew and St. John's Wort as reported by Murch and colleagues [8], we feel, may be present to counter the high level of free radicals generated from metabolic activities and/or photooxidation by direct exposure to sun light and ultra-violet light [12]. During photosynthesis and oxidative metabolism, oxygen is either liberated or consumed. Such reactions may generate a variety of reactive oxygen intermediates (ROI) which are toxic to cells. These toxic by-products can cause havoc in all organisms when no adequate strategies are provided for their detoxification. The high levels of melatonin observed in St. John's wort and feverfew, plants that thrive best in northernmost latitudes of America, might be related to melatonin's importance in retarding or blunting environmental stressors such as extremes in cold or heat, prolonged episodes of drought as well as protection against environmental chemical pollutants which may cause damage to the germ tissue residing in the seed or flower. Therefore, plants may utilize melatonin as part of their antioxidant defense system which also includes enzymes such as glutathione, peroxidase, catalase, photosynthetic pyridine nucleotide reductase and others [13,14].

Melatonin is a broad-spectrum antioxidant that exhibits both lipophilic and hydrophilic properties [15]. This property gives melatonin the capacity to enter all intracellular protoplasmic compartments and to protect all parts of plants, especially the germ and reproductive tissues which reside in the seeds and flowers, respectively, from oxidative damage and cell death.

Because of the many, unique physiological activities of melatonin observed in animal models, it is important to further investigate melatonin levels in plant tissues, especially in seeds and flowers which carry the germ tissue of the next generation. This study is aimed at identifying, quantifying, and verifying the presence of melatonin in the seeds of 15 edible plants using radioimmunoassay and HPLC.

Materials and methods

The seeds used in these experiments were obtained from Frontier Natural Products Corporation through their distributor Sun Harvest Farms (San Antonio, TX). Fifteen types of seeds were obtained: black and white mustard (*Brassica nigra* and *Brassica hirta*, respectively); fenugreek (*Trigonella faena-graecum*); milk thistle (*Silybum marianum*); celery (*Apium gravolens*); alfalfa (*Medicago sativa*); coriander (*Coriandrum sativum*); green cardamom (*Elettaria cardamomum*); fennel (*Foeniculum vulgare*); poppy (*Popaver somniferum*); anise (*Pimpinella anisum*); sunflower (*Helianthus annuus*); flax (*Linum usitatissimum*); almond (*Prunus amydalus*) and the Chinese wolfberry (*Lycium barbarum*).

To reduce bacterial and fungal growth in the products, the seeds were kept at 4°C until they were pulverized with a mortar and pestle using 3mL of cold ethanol (per 100 mg tissue) as the homogenizing solution. The homogenized plant tissue was centrifuged at 4°C, 10,000 rpm for 10 minutes. The supernatant was removed and saved in Eppendorf tubes. The ethanol was evaporated off under vacuum (20 psi). To the residue was added 1.2 mL of HPLC grade water and vortexed for 30 seconds. Two hundred fifty microliters of each sample, in duplicate, were used for each seed sample and melatonin was estimated using radioimmunoassay (RIA) previously documented [16].

For HPLC analysis, the residues were dissolved in 120 mL of the HPLC mobile phase. The samples were then filtered using 0.22 μm filter (millipore) and then centrifuged for 1 h at 15,000 g. Thirty microliters of each sample was injected into the HPLC-ECD system. The HPLC-ECD system was composed of an ESA 580 dual pump, 504 autosampler, and a Coularray 8 Channel coulometric array electrochemical detector. A C_{18} reversed-phase column was used on-line with a mobile phase of 0.1 M potassium phosphate buffer (pH 4.5) with acetonitrile (20%) at a flow rate of 1 ml/min. Applied potentials were initiated at 200 mV for channel one and was increased by 100 mV for each of the higher channels resulting in 900 mV at channel 8 [17].

Results

The melatonin recovery rate, using our protocol for extraction, was in the range of 20% representing a comparison of known melatonin concentration in samples before homogenization and extraction with cold ethanol. The recovery rate seems lower than a comparable vertebrate tissue samples because of the fibrous nature of the plant residues and the procedure used to pulverize the hardened seeds, which employed use of a mortar and pestle. Melatonin adhered to the fibers and the extraction utensils, thus, making the recovery rate lower. The seed melatonin concentration was identified and quantitated in 15 edible seeds using RIA. The immunoreactive melatonin was later confirmed as melatonin using the HPLC-ECD method. Melatonin was observed in every seed of the plants tested. The highest physiological level of melatonin was found in the white mustard (*B. hirta*), with the black mustard (*B. nigra*) having the next highest concentration. The amount of melatonin in the seeds ranged from about 2 to 190 ng/g dry weight of seed. A HPLC spectrum of seed melatonin from the fenugreek sample is presented in Fig. 1. The concentration of melatonin in each seed sample is summarized in Table 1. The lowest concentration of melatonin was observed in the milk thistle (*Silybum marianum*).

Discussion

This study was aimed first at identifying, then confirming the concentration of melatonin in the seeds of 15 edible plants. Without exception, the results show high levels of melato-

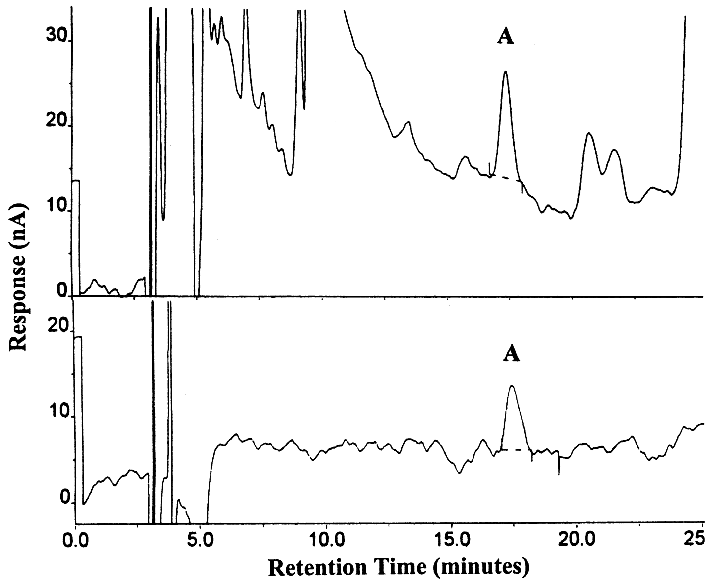

Fig. 1. HPLC spectra of melatonin from Fenugreek seeds and the melatonin standard. The retention time of melatonin in this system was roughly 17.2 minutes. “A” in the upper panel represents the peak of melatonin in Fenugreek seeds, while the “A” in the lower panel represents the spectrum of the melatonin standard (100 pg).

nin in seeds. Thus, consumption of plant tissues containing could enhance circulating melatonin levels in the blood and also in the gastro-intestinal tract [18–20]. The results of our study clearly provide convincing evidence that the seeds of plants contain large amounts of melatonin.

Table 1
Melatonin concentrations in seeds of 15 edible plants

Common name	Scientific name	Melatonin content (ng/g dry seed)
Milk thistle	*Silybum marianum*	2
Poppy	*Popaver somniferum*	6
Anise	*Pimpinela anisum*	7
Coriander	*Coriandrum sativum*	7
Celery	*Apium graveolens*	7
Flax	*Linum usitatissimum*	12
Green cardamom	*Elettaria cardamomum*	15
Alfalfa	*Medicago sativum*	16
Fennel	*Foeniculum vulgare*	28
Sunflower	*Helianthus annuus*	29
Almond	*Prunus amygdalus*	39
Fenugreek	*Trigonella foenum-graecum*	43
Wolf berry	*Lycium barbarum*	103
Black mustard	*Brassica nigra*	129
White mustard	*Brassica hirta*	189

Large amounts of melatonin were particularly evident in the white and black mustard, wolfberry, and fenugreek seeds. There was considerable variation in the melatonin content in the seeds we studied. For example, the lowest concentration of melatonin was found in the milk-thistle (2 ng dry seed), whereas the highest concentration of melatonin was observed in the white mustard (189 ng/g). But the level of melatonin in milk-thistle is still very high relative to levels found in vertebrate blood [9]. The reason for the considerable variation in the melatonin content of seeds examined may probably be due to differences in the water content of the seeds, the abiotic environment in which the seeds were grown and intrinsic physiological and genetic variability related to each species. Coincidentally, we also investigated the fruit of the wolfberry and the flower of the St. John's wort and found that both contained 10-fold higher melatonin concentrations than found in the wolfberry seed (L.C. Manchester, unpublished observations). The significance of these high levels of melatonin in seeds, fruits and flowers is not known. Melatonin may serve a protective role in plants that is similar to the many well documented studies in vertebrates [10] which melatonin reduced oxidative damage to macromolecules such as lipids, proteins, and DNA. These damaged parameters are measures of the levels of oxidative stress [15,20].

The discovery of melatonin as a scavenger of hydroxyl radicals [21] along with the observation that melatonin is ubiquitously distributed throughout the plant and animal kingdoms suggest that the role of melatonin as a scavenger may be conserved from plants through higher vertebrate organisms. If this is the case, then melatonin's role as a transducer of photoperiodic cues may be a more recently evolved function in certain organisms. Melatonin's primary role in plants may be related to scavenging free radicals and protecting the germ and reproductive tissues from biological and chemical assaults. The seeds and flowers of plants represent the anlage of the next generation of a plant. They contain the reproductive and germ tissues necessary for a plant's survival amidst a hostile environment enriched with ultraviolet light, chemical toxins and reactive toxicants. Thus, the presence of melatonin in seeds, fruits, and flowers may be required in high concentrations to offset the damage associated with free radical generation that results from reactive intermediates, toxins, and pollutants.

Melatonin has both direct and indirect mechanisms for reducing and neutralizing free radicals and reactive oxygen intermediates [15]. Besides functioning as a direct free radical scavenger in mammals, it also stimulates several antioxidative enzymes including glutathione peroxidase and reductase [13, 14]. Melatonin also inhibits the activity of the prooxidative enzyme, nitric oxide synthase. There is some evidence that melatonin serves as a chelator of transition metals [22] thus preventing macromolecular dysfunction while maintaining functional integrity of the phospholipids and proteins of membranes [23]. The seeds of plants contain lipids which can readily be oxidized; the accumulation of such products compromises the germination potential of the seed. Melatonin's ability to permeate all tissues and subcellular compartments makes it an ideal protector of cells from lipid peroxidation [24, 25], protein damage [26, 27], and DNA destruction [28]. Free radicals, reactive oxygen intermediates, ultraviolet light, and toxic chemicals in the environment cause apoptosis and cell death in plants as in animals.

The destruction caused by free radicals affect virtually all macromolecules including both nuclear and mitochondrial DNA. In mammalian cells, melatonin enters the nucleus [29]. Assuming this is also true in plants, melatonin would be in a position to prevent free radical attacks on nuclear DNA; as a result it may offer on-site protection to the genome and thus prevent subsequent generations of plants. Finally, certain plants such as the alpine penny-cress have a high tolerance for toxic metals extracted from contaminated soils. The resistance of some of these plants to toxicants may relate to their intrinsic levels of melatonin. Although there is currently no evidence showing that plants that survive in metal-contaminated soils have higher than usual melatonin levels, nevertheless, since melatonin is a proven antioxidant and free radical scavenger, it would likely increase the resistance of plants to agents which generate free radicals and reactive intermediates.

In conclusion, the presence of melatonin in high levels in several different edible plant seeds suggests a primary role as part of the antioxidative defense system which is geared towards protecting germ and reproductive tissues from biological and environmental assaults. Melatonin, from seeds and other plant residues, is transported across the gastrointestinal mucosa into the vasculature where it raises plasma melatonin. Hattori and coworkers [6] have identified high levels of plasma melatonin after fasted chickens were fed plant tissues known to have high levels of melatonin.

Acknowledgments

This study was supported in part by a Faculty Development Fellowship. We are indebted to St. Mary’s University, Dean Anthony J. Kaufmann, Ph.D, and the Faculty Development Committee. We also acknowledge the assistance of the Tri-Beta Biological Honor Society for the critique of the manuscript.

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