The consumption of a Jerte Valley cherry product in humans enhances mood, and increases 5-hydroxyindoleacetic acid but reduces cortisol levels in urine

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A B S T R A C T

Purpose: Jerte Valley cherries contain high levels of tryptophan, serotonin, and melatonin. These molecules have been shown to be involved in mood regulation. It has been suggested that a complex inter-relationship between brain serotonin, circulating levels of cortisol (the major stress hormone), and the hypothalamic–pituitary–adrenal axis exists in the regulation of stress responses, where cortisol and serotonin act as markers of mood disturbances. Moreover, there is growing evidence that altered HPA activity is associated with various age-related pathologies. The present study evaluated the effect of the ingestion of a Jerte Valley cherry-based product, compared to a placebo product, on urine cortisol and 5-hydroxyindoleacetic acid (5-HIAA) levels, and on mood in young, middle-aged, and elderly participants.

Methods: Cortisol and 5-HIAA acid levels were measured by commercial enzyme-linked immunosorbent assay kits. The mood state profile was assessed using a visual analogue scale and the state–trait anxiety inventory.

Results: Our findings showed that the ingestion of the Jerte Valley cherry product decreased urinary cortisol and increased urinary 5-HIAA levels in all the experimental groups. Moreover, the cherry product was able to lessen anxiety status in the middle-aged and elderly participants, and enhanced subjective mood parameters, particularly family relationships in young participants, and frame of mind and fitness in both middle-aged and elderly subjects.

Conclusions: The consumption of the Jerte Valley cherry product may protect against stress and act as a mood enhancer by increasing serotonin availability to the organism, particularly with advancing age.

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1. Introduction

Globally, it is estimated that as many as 450 million people suffer from mood disturbances, with depression and anxiety being among the most prevalent. As an example, 13.6% of the population in six European countries, including Spain, reported having had an anxiety disturbance during their lifetime (Alonso et al., 2004), and more than 150 million people suffer from depression at any given point in time.

The limbic hypothalamus–pituitary–adrenal (HPA) axis is a central control and regulatory system of the organism that connects the central nervous system (CNS) with the hormonal system, thereby conforming a stress-responsive neuroendocrine system (Kudielka and Kirschbaum, 2005). It is widely accepted that the activated HPA axis not only regulates body peripheral functions, but also has profound effects on the brain (Pariante and Lightman, 2008). Many patients with depressive and/or anxiety disturbances show disruptions in HPA axis reactivity as indexed by increased baseline plasma cortisol levels compared with healthy controls (Rajewska and Rybakowski, 2003; Vreeburg et al., 2009). However, not only the HPA axis, but also brain neuronal systems, including the monoaminergic systems, in particular the serotonergic system, play an important role in the regulation of many physiological and behavioural processes including mood. In this sense, a strong relationship between the HPA axis and the serotonergic system has been reported (Porter et al., 2004). Stress-induced elevated cortisol reduces the tonic level of serotonin in the synaptic cleft and stimulates its reuptake after a neuronal impulse, thereby leading to defective serotonergic neurotransmission in the CNS (Tafet et al., 2001) as has been reported in depression (Duval et al., 2001).

Ageing can be defined as a progressive decline in physiological efficiency regulated by extremely complex multifactorial processes.
Several studies provide evidence that hyperactivity of the HPA axis contributes to the neuronal and peripheral deterioration associated with ageing (Ferrari and Magri, 2008), which is reflected in decreased concentrations of the neurotransmitter serotonin, a modulator of ageing in the brain (Siblele et al., 2007). Moreover, there is clear evidence that ageing is associated with elevated basal morning levels of circulating glucocorticoids such as cortisol (Tizabi et al., 1992). Overall, there is increasing evidence that altered HPA activity, e.g., increased glucocorticoid activity or a decreased brain serotonin concentration, is associated with several age-related pathologies (Aguiera, 2011).

The amino acid tryptophan, which is obtained from the diet in humans, is the direct precursor of serotonin. It has been shown that increases in plasma tryptophan availability enhance positive mood and dampen the cortisol response after an acute experimental stress exposure in stress-vulnerable subjects by enhancing brain serotonin mechanisms that are involved in adaptation to stress (Firx and Markus, 2009; Markus et al., 2000). For this reason, several studies have focused on evaluating the efficacy of the consumption of tryptophan-enriched diets to reduce cortisol responses and improve the ability to cope with stress, probably by way of counteracting alterations in brain serotonin (Lepage et al., 2002). However, most of these studies were carried out on animals, while available information on humans regarding the effect of tryptophan-enriched diets on both the ability to cope with stress, probably by way of counteracting alterations in brain serotonin (Lepage et al., 2002). However, most of these studies were carried out on animals, while available information on humans regarding the effect of tryptophan-enriched diets on both stress-induced cortisol responses and mood is scarce.

Cherries are an important source of phytochemicals and reportedly have important health-promoting properties, including antioxidant effects (McCune et al., 2011). In this regard, Jerte Valley sweet cherries contain not only high concentrations of anthocyanin pigments and phenolic compounds (González-Gómez et al., 2010), but also substantial amounts of melatonin, serotonin (González-Gómez et al., 2009), and tryptophan (Cubero et al., 2010). Also, it has been reported that both a Jerte Valley cherry-enriched diet (Garrido et al., 2010) and the ingestion of a Jerte Valley cherry-based product (Garrido et al., 2009) exhibit sleep-promoting actions, and increase both urinary 6-sulfatoxymelatonin (aMT6-s) and the antioxidant status in humans. Therefore, the purpose of this study was to compare the effect of the ingestion of a Jerte Valley cherry-based product (patent no. ES 2342141 B1), compared to a placebo product on cortisol and 5-hydroxyindoleacetic acid (5-HIAA) urinary levels and on mood, in young, middle-aged, and elderly subjects.

2. Material and methods

2.1. Participants

The study was carried out in young (20–30 years old, n = 10; 5 men and 5 women), middle-aged (35–55 years old, n = 10; 5 men and 5 women) and elderly (65–85 years old, n = 10; 5 men and 5 women) volunteers whose weight, height, and body mass index (BMI) values are presented in Table 1. The study was approved by the Ethics Committee of the University of Extremadura (Badajoz, Spain) in accordance with the Declaration of Helsinki, the Council of Europe, and the Universal Declaration of UNESCO on human rights, biomedicine, and human genome. All participants were of Caucasian ethnicity and were recruited through word of mouth. There were no dropouts during the study. Each participant was ascertained to be in good health from their medical history and a clinical examination including routine laboratory tests and screening. The participants were non-smokers, were not using any medication, and abstained from alcohol. Informed consent was obtained from all participants.

2.2. Experimental design

The study had a blind, placebo-controlled, randomised, crossover design with two treatment periods of five days each, separated by a washout period of one week. Either the placebo or the Jerte Valley cherry product was consumed twice a day, as lunch and dinner desserts. Each dose of cherry product (27.85 g) consisted of 18.85 g of pitted, freeze-dried cherries (equivalent to 141 g fresh cherries) in equal parts of four Jerte Valley cherry cultivars (Bourlat, Navalinda, Pico Negro, and Pico Colorado), plus 7.5 g maltodextrin and 1.5 g ascorbic acid (Spanish patent no. ES 2342141 B1). The freeze-dried, cherry-based product was then ground to a powder, and then diluted in water and bottled in 12 fl oz plastic bottles containing 125 ml (4.22 fl oz) of cherry-based product per dose. One dose of the product provided roughly 1580 mg phenolic compounds (expressed as gallic acid equivalents), 30 mg anthocyanins (calculated as malvidin equivalents), 690 mg total antioxidant capacity (TAC, expressed as Trolox equivalents), 2 mg tryptophan, 27 ng serotonin, and 16 ng melatonin.

The placebo was a commercial cherry-flavoured soft drink (Kool-Aid®, Kraft Foods, USA; ingredients listed: citric acid, salt, calcium phosphate, red 40, artificial flavour, ascorbic acid, artificial colour, blue 1) which was prepared by mixing it with water in the proportion recommended by the manufacturer, followed by bottling in 12 fl oz plastic bottles to contain 125 ml (4.22 fl oz) of placebo product per dose.

2.3. Cortisol and 5-hydroxyindoleacetic acid (5-HIAA) urine levels

First‐void morning urine and urine at 20:00 h were collected before the trial (basal values), after a 5‐day intake of the Jerte Valley cherry-based product or the placebo (trial values), and one day following its termination (post-trial values). The samples were stored at −20 °C until biochemical assay.

Cortisol levels were measured in both urine samples and 5-HIAA levels were measured in the 20:00 h urine using commercial enzyme‐linked immunosorbent assay kits from DRG Diagnostics (Marburg, Germany) and IBL International (Hamburg, Germany), respectively, following the manufacturers’ instructions. To adjust for variation in the dilution of urine, cortisol and 5-HIAA were expressed as urine cortisol/creatinine or urine 5-HIAA/creatinine ratios, respectively. The creatinine concentration was determined by means of the Jaffe’s test.

2.4. Mood state profile

Changes in mood were measured on a visual analogue scale (VAS). These scales have been found to be effective tools in measuring changes over time in response to treatment for symptoms of mood disturbance, and their reliability and validity have been well documented (Baeken et al., 2008; McCormack et al., 1988; Mosimann et

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Weight, height, and body mass index (BMI) of young, middle-aged, and elderly participants.</th>
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<tbody>
<tr>
<td></td>
<td>Weight</td>
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<tr>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>Young</td>
<td>81.00 ± 2.70</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>1.80 ± 0.04</td>
</tr>
<tr>
<td>Elderly</td>
<td>24.97 ± 2.84</td>
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</tbody>
</table>

Each value represents the mean ± SD of five participants.
The mood visual analogue scale was applied for subjective assessment in the form of a 100-mm horizontal line oriented with anchors placed at both poles, indicating antithetic conditions. Four such lines were employed (4 × 100-mm VAS) to measure the volunteers’ general mood status in terms of ‘family relationships’, ‘social relations’, ‘frame of mind’, and ‘fitness’. Participants were asked to rate how they were feeling on these 100-mm scales in which the descriptors ranged from ‘very bad’ (0 mm) to ‘very good’ (100 mm).

The second measurement instrument used was the state–trait anxiety inventory (STAI) (Scholey et al., 2009). This comprises two subscales each containing 20 statements (e.g. ‘I am calm’). The state anxiety subscale measures the anxiety at the moment of scoring, while the trait anxiety subscale measures dispositional anxiety or anxiety in general. Participants rated how much they agreed with each statement by marking a 4-point scale ranging from ‘not at all’ to ‘very much so’ on the state subscale, and from ‘hardly ever’ to ‘almost always’ on the trait subscale. Scores ranged from 20 to 80, with higher scores indicating more anxiety.

Participants completed both tests before the beginning of the trial (basal score), after the 5-day intake of the Jerte Valley cherry product or the placebo (trial scores), and one day following its termination (post-trial scores). Up to 10 min was allowed for completion of these paper and pencil surveys.

2.5. Statistical analysis

The Kolmogorov–Smirnov test was applied to test for normality of the distribution of the results. When normality could be assumed (STAI and VAS scores), parametric methods were employed, i.e. analysis of variance (ANOVA) for repeated measures, to examine changes in each dependent variable from baseline. If significant main effects were detected, Tukey’s post-hoc tests were performed to determine which points differed significantly from baseline. When data did not fulfill a normal distribution (cortisol and 5-HIAA urine levels), Friedman’s non-parametric test was performed to examine changes in each dependent variable from baseline. If significant main effects were detected, Dunn’s post-hoc tests were carried out to determine which points differed significantly from baseline. Regarding urinary cortisol levels, since samples were assessed in the morning and in the evening, data were also analysed taking into account time of day (morning, evening) using a two-way [intervention (placebo, cherry product) × time-of-day] general linear model ANOVA with both variables as repeated measure factors.

Values are expressed as mean ± SEM. The significance level was set at p < 0.05. F- or χ²-values (for the main analysis), and q- or t-values (for post-hoc tests) were also reported. Additionally, effect sizes were computed for relevant change-from-baseline VAS scores using the Cohen’s d statistic (difference between means divided by pooled standard deviation). All analyses were performed using GraphPad Prism (version 5.0, 2007; GraphPad Software, Inc; San Diego, CA).

3. Results

Table 2A presents the scores obtained on the VAS test. There were significant main effects of intervention on the majority of mood items, i.e., it was observed that the intake of the cherry product generally improved mood in all three age groups. The young participants in particular improved their self-rated family relationships after the intake of the cherry product [92.5 ± 4.8 mm; F(9, 18) = 6.93; q(9) = 5.16; p < 0.05]. The self-rated frame of mind was enhanced in the middle-aged participants in both the trial [89.4 ± 5.0 mm; F(9, 18) = 22.97; q(9) = 8.56; p < 0.05] and post-trial [88.2 ± 5.2 mm; F(9, 18) = 22.97; q(9) = 8.01; p < 0.05] conditions with respect to their basal scores. Also, this group’s scores in relation to fitness were improved in both the trial [83.2 ± 9.5 mm; F(9, 18) = 12.15; q(9) = 5.96; p < 0.05] and post-trial [83.7 ± 9.2 mm; F(9, 18) = 12.15; q(9) = 6.11; p < 0.05] conditions with respect to their basal scores. Finally, the elderly participants improved their self-rated frame of mind [92.1 ± 4.2 mm; F(9, 18) = 4.75; q(9) = 3.99; p < 0.05] and their fitness [93.6 ± 3.0 mm; F(9, 18) = 6.14; q(9) = 4.71; p < 0.05] with respect to their corresponding basal values after the intake of the product. The enhanced fitness persisted one day after the end of the trial [88.1 ± 7.8 mm; F(9, 18) = 6.14; q(9) = 3.67; p < 0.05]. As shown in Table 2B, the 4 × VAS scores after the intake of the placebo remained unchanged in all three age groups, thus providing supporting evidence that the changes reported in the participants’ mood were likely to have been due to the 5-day intake of the Jerte Valley cherry product.

The ingestion of the cherry product had significant main effects of intervention on the anxiety of the middle-aged and elderly participants, as assessed by the STAI scales (Fig. 1). However, there was no significant effect of intervention on anxiety measures of young subjects. After the intake of the product, the state anxiety scores (Fig. 1A) were significantly lower than their corresponding basal scores in both the middle-aged [32.7 ± 3.0; F(9, 36) = 5.60; q(9) = 4.44; p < 0.05] and the elderly [32.4 ± 3.2; F(9, 36) = 6.35; q(9) = 5.13; p < 0.05] groups. Likewise, the ingestion of the product caused a significant decrease (p < 0.05) in trait anxiety scores (Fig. 1B) in both the middle-aged [32.5 ± 4.4; F(9, 36) = 4.76; q(9) = 4.85] and elderly [32.5 ± 4.1; F(9, 36) = 6.09; q(9) = 4.61] groups compared with their corresponding basal scores. The effect continued in post-trial conditions for middle-aged [35.6 ± 4.6; F(9, 36) = 4.76; q(9) = 4.52; p < 0.05] and elderly [31.8 ± 4.3; F(9, 36) = 6.09; q(9) = 4.20; p < 0.05] participants. Anxiety scores obtained after the intake of the placebo remained unchanged throughout the trial, thereby providing supporting evidence that the beneficial effects on anxiety were likely to have been due to the 5-day intake of the product.

Fig. 2 shows the urine cortisol levels obtained after the intake of a Jerte Valley cherry-based product or the placebo in the young, middle-aged, and elderly participants. Since samples were assessed in the morning and in the evening, the data were analysed taking into account time of day (morning, evening) using a two-way lineal model ANOVA (intervention × time-of-day) with both variables as repeated measure factors. This revealed a significant main effect of intensity on cortisol levels in young [F(1, 117) = 42.30; p < 0.05], middle-aged [F(1, 117) = 15.80; p < 0.05] and elderly [F(1, 117) = 35.27; p < 0.05] participants. To explore this effect further, within-subject Friedman tests were conducted for the morning and the evening measures separately. Thus, the consumption of the product led to decline (p < 0.05) in the urine cortisol levels in both first-void urine [morning; 0.66 ± 0.15; χ²(8) = 45.31; (9) = 4.53] and 20:00 h urine [evening; 0.64 ± 0.12; χ²(8) = 45.31; (9) = 6.98] in young participants with respect to their basal values (Fig. 2A). A similar effect was observed 1 day after the termination of the cherry product intake, with the decrease being statistically significant in urine collected in the evening [0.70 ± 0.14; p < 0.05; χ²(8) = 45.31; (9) = 5.10]. Also, a significant decrease was found after the ingestion of the product in urine collected from middle-aged volunteers (Fig. 2B), in both first-void [0.59 ± 0.06; p < 0.05; χ²(8) = 43.59; (9) = 13.03] and 20:00-h urinary samples [0.53 ± 0.11; p < 0.05; χ²(8) = 43.59; (9) = 10.15], with respect to their basal values. Thus, both morning [1.02 ± 0.15; χ²(8) = 43.59; (9) = 6.25] and evening [1.06 ± 0.22; χ²(8) = 43.59; (9) = 5.70] urine cortisol levels raised, reaching their respective basal values 1 day after the end of the trial (p < 0.05 vs. its corresponding trial value). In the elderly group (Fig. 2C), the ingestion of the cherry product diminished the urine cortisol levels contained in both the morning [0.47 ± 0.13; p < 0.05; χ²(8) = 44.80; (9) = 8.95] and the evening [0.66 ± 0.05; p < 0.05; χ²(8) = 44.80; (9) = 11.87] urine. The post-trial urine cortisol values remained unchanged in the elderly group. This effect was statistically significant [F(1, 117) = 15.80; p < 0.05].
Table 2A
Effect of the Jerte Valley cherry product on self-rated scores obtained on a 4× VAS scale (family relationships, social relations, frame of mind, and fitness) in basal (before the intake of the cherry product), trial (after the intake of the cherry product for 5 consecutive days), and post-trial (one day after the end of the trial) conditions in young, middle-aged, and elderly participants. Results are expressed in millimetres (mm). Each value represents the mean ± SEM of ten participants.

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Middle-aged</th>
<th>Elderly</th>
<th></th>
<th>Young</th>
<th>Middle-aged</th>
<th>Elderly</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Trial</td>
<td>Post-trial</td>
<td>Basal</td>
<td>Trial</td>
<td>Post-trial</td>
<td>Basal</td>
<td>Trial</td>
</tr>
<tr>
<td>Family relationships (mm)</td>
<td>85.2 ± 6.8</td>
<td>92.5 ± 4.8* (0.81)*</td>
<td>90.8 ± 6.1 (0.62)</td>
<td>86.5 ± 8.9</td>
<td>89.8 ± 6.1 (0.26)</td>
<td>91.2 ± 6.9 (0.37)</td>
<td>85.9 ± 8.4</td>
<td>84.5 ± 7.6 (0.13)</td>
</tr>
<tr>
<td>Social relations (mm)</td>
<td>83.3 ± 5.4</td>
<td>84.8 ± 7.1 (0.19)</td>
<td>87.2 ± 3.8 (0.51)</td>
<td>86.1 ± 6.6</td>
<td>84.5 ± 9.0 (0.12)</td>
<td>87.5 ± 7.6 (0.12)</td>
<td>88.8 ± 4.7</td>
<td>88.9 ± 6.5 (0.01)</td>
</tr>
<tr>
<td>Frame of mind (mm)</td>
<td>76.8 ± 13.4</td>
<td>82.0 ± 12.8 (0.24)</td>
<td>82.2 ± 8.5 (0.29)</td>
<td>72.0 ± 11.2</td>
<td>89.4 ± 5.0 (1.21)</td>
<td>88.2 ± 5.2 (1.12)</td>
<td>78.2 ± 10.2</td>
<td>82.1 ± 4.2* (1.08)</td>
</tr>
<tr>
<td>Fitness (mm)</td>
<td>70.3 ± 14.0</td>
<td>79.4 ± 8.7 (0.47)</td>
<td>80.8 ± 6.8 (0.58)</td>
<td>70.8 ± 10.4</td>
<td>83.2 ± 9.5* (0.80)</td>
<td>83.7 ± 9.2* (0.82)</td>
<td>79.2 ± 12.4</td>
<td>93.6 ± 3.0* (0.96)</td>
</tr>
</tbody>
</table>

*p < 0.05 with respect to their corresponding basal scores.

* Effect sizes (Cohen's d) are shown in parentheses for change-from-basal scores.

Table 2B
Effect of the placebo on self-rated scores obtained on a 4× VAS scale (family relationships, social relations, frame of mind, and fitness) in basal (before the intake of the placebo), trial (after the intake of the placebo for 5 consecutive days), and post-trial (one day after the end of the trial) conditions in young, middle-aged, and elderly participants. Results are expressed in millimetres (mm). Each value represents the mean ± SEM of ten participants.

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Middle-aged</th>
<th>Elderly</th>
<th></th>
<th>Young</th>
<th>Middle-aged</th>
<th>Elderly</th>
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<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Trial</td>
<td>Post-trial</td>
<td>Basal</td>
<td>Trial</td>
<td>Post-trial</td>
<td>Basal</td>
<td>Trial</td>
</tr>
<tr>
<td>Family relationships (mm)</td>
<td>79.8 ± 6.1</td>
<td>81.4 ± 5.9 (0.28)*</td>
<td>82.0 ± 5.7 (0.39)</td>
<td>90.5 ± 5.0</td>
<td>88.5 ± 5.6 (0.40)</td>
<td>88.3 ± 4.9 (0.47)</td>
<td>88.4 ± 6.1</td>
<td>82.2 ± 12.9 (0.45)</td>
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<tr>
<td>Social relations (mm)</td>
<td>84.2 ± 5.4</td>
<td>81.8 ± 3.5 (0.41)</td>
<td>83.4 ± 3.1 (0.18)</td>
<td>83.3 ± 1.7</td>
<td>81.6 ± 6.1 (0.40)</td>
<td>82.0 ± 3.6 (0.49)</td>
<td>87.0 ± 8.6</td>
<td>78.8 ± 12.6 (0.54)</td>
</tr>
<tr>
<td>Frame of mind (mm)</td>
<td>84.6 ± 5.3</td>
<td>82.2 ± 10.4 (0.25)</td>
<td>86.6 ± 6.2 (0.37)</td>
<td>79.3 ± 5.7</td>
<td>79.0 ± 4.9 (0.06)</td>
<td>82.3 ± 3.9 (0.50)</td>
<td>66.2 ± 9.5</td>
<td>64.0 ± 9.1 (0.25)</td>
</tr>
<tr>
<td>Fitness (mm)</td>
<td>78.2 ± 5.6</td>
<td>76.2 ± 8.2 (0.30)</td>
<td>75.6 ± 11.0 (0.31)</td>
<td>78.3 ± 1.9</td>
<td>76.6 ± 3.3 (0.43)</td>
<td>76.6 ± 6.0 (0.33)</td>
<td>67.5 ± 13.0</td>
<td>69.2 ± 12.9 (0.14)</td>
</tr>
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</table>

* Effect sizes (Cohen's d) are shown in parentheses for change-from-basal scores.
lower than the basal levels, although not significantly. Interestingly, the placebo intake did not modify cortisol levels from either the morning or the evening urines in any experimental group (Fig. 2A, B, and C).

Fig. 3 shows the 5-HIAA levels found in the 20:00 h urine collected after a 5-day intake of the product or the placebo and 1 day afterwards in each experimental group. Once again, analyses of changes from baseline values indicated that the intervention was generally effective. In fact, the Jerte Valley cherry product intake produced a rise in 5-HIAA levels in the young \[1.39 \pm 0.10; \chi^2(5) = 28.60; t(9) = 9.60\], middle-aged \[2.49 \pm 0.42; \chi^2(5) = 28.20; t(9) = 6.20\] and elderly \[1.62 \pm 0.06; \chi^2(5) = 29.92; t(9) = 24.75\] participants with respect to their corresponding basal values. Regarding the post-trial values, the 5-HIAA levels diminished in all groups of age, the effect being particularly significant in elderly participants whose 5-HIAA levels substantially decreased \[0.87 \pm 0.06; \chi^2(5) = 29.92; t(9) = 22.87\] with respect to their trial values. The placebo intake did not change the 5-HIAA levels in any group.

4. Discussion

In the present work, it was observed that the intake of a Jerte Valley cherry product had beneficial effects on the mood of healthy participants. Compared with the placebo, which did not have any effect on the parameters evaluated, the cherry product reduced anxiety (as measured in terms of both state and trait anxiety) and had a positive effect on subjective mood (as measured in terms of family relationships, social relations, frame of mind, and fitness). This was especially notable in the middle-aged and elderly participants. These findings were supported by the decrease in urine cortisol and the increase in 5-HIAA concentrations found after the cherry product consumption.

Mental health is crucial to the overall well-being of individuals, societies, and countries, but the human brain is exposed to frequent challenges as a consequence of stress. Although the stress response of the body is meant to maintain stability or homeostasis, long-term activation of the stress system can have a harmful or even lethal effect on the body, increasing the risk of depression, anxiety, and a variety of other disruptions. A major focus of investigation has been the role of the HPA axis, both as a marker of the stress response and as a mediator of additional downstream pathophysiological changes in the brain (Klok et al., 2011; Mello et al., 2003). Several studies in humans and experimental animals provide evidence that hyperactivity of the HPA axis contributes to the neuronal and peripheral deterioration associated with ageing (Aguiera, 2011). In advancing age, the inability to limit the stress response, especially during chronic stress, is likely to enhance the effects of ageing due to the damaging effects of prolonged exposure to stress hormones (Aguiera, 2011).

Mental ageing itself may be due to loss of neuronal and synaptic function and a decrease in concentrations of various neurotransmitters (Slotkin et al., 2005). Indeed, it has been reported that the ageing process results in a progressive decline in brain serotonin levels, and in changes in serotonin metabolism and in the expression of many serotonin receptors (Duncan and Hensler, 2002; Duncan et al., 2000). This could be responsible for alterations in many of the organism’s physiological and behavioural functions, including mood and sleep (Clareglia et al., 2011).

In recent years, there has been great interest in determining the role of compounds of plant origin, i.e., phytonutrients, in improving health. Despite the mechanisms of such protection not being fully understood, and the plethora of potentially beneficial ingredients that exist in plant foods, there is relatively consistent evidence that the antioxidant and anti-inflammatory properties of phytonutrients play a role in this protection (Heber, 2004; Mayorga et al., 2004). In particular, both sweet and tart cherries are an excellent source of nutrient and bioactive food components for the human diet (Garrido et al., 2009; Garrido et al., 2010), and several studies have shown their beneficial effects on cancer, cardiovascular disease, diabetes, and inflammation (McCune et al., 2011). Some experiments have been carried out to evaluate the effects of the consumption of tart cherry juice on health (Connolly et al., 2006; Kuehl et al., 2010; Traustadottir et al., 2009), since it has been reported that natural antioxidants found in...
These fruits may be used as ingredients of functional foods (Blando et al., 2004). However, none of these studies attributed the aforementioned health-promoting effects to the presence of tryptophan, serotonin, and/or melatonin in the cherries.

In the present work, the improvement of anxiety and mood state found after the intake of the Jerte Valley cherry product was likely due to the elevation in the participants’ circulating levels of serotonin, serotonin, and melatonin. Indeed, the supplementation of tryptophan, serotonin, and/or melatonin in the diet has been reported to have beneficial effects on mood, and to present antidepressant-like actions (Rios et al., 2010; Silber and Schmitt, 2010). These findings are also consistent with previous work which demonstrated that improving the tryptophan supply in the diet or applying a melatonin treatment results in an increase of serotonin and melatonin circulating levels in both diurnal and nocturnal animals (Mateos et al., 2009; Paredes et al., 2009).

There has been a clinical interest in the measurement of cortisol because disturbances of cortisol levels are evident in many pathological states. Cortisol has long been used in human psychobiological studies as a biological marker of stress, anxiety, and depression (Levine et al., 2007). Its effects on mood appear to be dose-dependent, since a transient moderate elevation in cortisolaemia due to acute stress has a protective effect on mood in critical situations (Het and Wolf, 2007), although permanent hypercortisolism may induce behavioural, psychic, and cognitive disturbances due to functional and, over time, structural alterations in specific target areas of the brain (Fietta et al., 2009). Thus, several studies have reported increased baseline plasma cortisol levels in depressed patients compared with healthy controls (Rajewska and Rybakowski, 2003), while others have found that more than half the cases of major depression are associated with hypercortisolism (Pitts et al., 1995). The present work has shown decreased urine cortisol levels after the intake of the Jerte Valley cherry product in all the participants (young, middle-aged, and elderly). This finding is particularly relevant for older populations since high basal levels of cortisol and loss of circadian rhythmicity have been associated with greater cognitive decline as age advances, and may have damaging effects that contribute to pathologies associated with advancing age, including depression, anxiety, and immune and metabolic disturbances (Aguilera, 2011). Although circulating cortisol was here measured indirectly by a non-invasive method, urinary cortisol levels have been extensively evaluated in both developmental (Diego et al., 2004) and affective research (Maes et al., 1998), and its clinical relevance has been clearly demonstrated (Lin et al., 1997). Likewise, it has been reported that urine cortisol excretion resulting from glomerular filtration is a useful index of integrated 24-h plasma free cortisol (Levine et al., 2007).

The VAS and STAI tests are psychological scales used as reliable tools for investigating changes in mood (Schaller et al., 2011; Scholey et al., 2009). The VAS test is by itself a suitable and valid method for measuring anxiety, and compares well with the state anxiety scores of the STAI test (Kindler et al., 2000). In the present study, lower cortisol levels were accompanied by an improvement in the subjective mood and anxiety scores as measured by both the VAS and the STAI tests, thereby underlining the potential of this cherry-based drink for use as a natural product with mood-enhancing properties, especially in middle-aged and elderly individuals. Furthermore, the 5-HIAA levels, the main urine metabolite of serotonin, were increased after the ingestion of the cherry product in all the experimental groups. Consequently, this indicates that brain and/or circulating serotonin levels were likely to have been elevated, since tryptophan supplementation produces an increase of these levels (Mateos et al., 2009; Paredes et al., 2009). In this respect, the elevated 5-HIAA levels may also be related to the mood improvement observed after the intake of the cherry product, since the neurotransmitter serotonin is involved in mood regulation (Murphy et al., 2008). For instance, studies of untreated depressed patients suggest that serotonin function is reduced in depression, and studies of treated patients indicate that the mechanism of action of antidepressants is mediated by the enhancement of serotonin and/or noradrenaline neurotransmission (Toker et al., 2010). Also, brain serotonin synthesis is considered to be proportional to tryptophan transport into the brain (Pardridge, 1998), and tryptophan depletion is found to reduce the rate of serotonin synthesis by 90% of baseline values (Young et al., 1999).

Since humans cannot synthesise tryptophan, it is considered as an essential amino acid that can only be obtained in the diet. In this respect, the ingestion of foodstuffs able to enhance not only tryptophan, but also serotonin and/or melatonin availability in the organism could induce an increase in the circulating levels of the amino acid, the neurotransmitter, and the indole, subsequently improving those functions in which deficiencies in these compounds have been reported, such as age-related sleep or behavioural disturbances.
Therefore, the consumption of a Jerte Valley cherry product (Spanish patent no. ES 2342141 B1) may protect against stress and act as a mood enhancer by improving serotonin availability in the organism.

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