Estrous cycle affects the neurochemical and neurobehavioral profile of carvacrol-treated female rats

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A B S T R A C T

Carvacrol is the major constituent of essential oils from aromatic plants. It showed antimicrobial, anticancer and antioxidant properties. Although it was approved for food use and included in the chemical flavorings list, no indication on its safety has been estimated. Since the use of plant extracts is relatively high among women, aim of this study was to evaluate carvacrol effects on female physiology and endocrine profiles by using female rats in proestrus and diestrus phases. Serotonin and metabolite tissue content in prefrontal cortex and nucleus accumbens, after carvacrol administration (0.15 and 0.45 g/kg p.o.), was measured. Drug effects in behavioral tests for alterations in motor activity, depression, anxiety-related behaviors and endocrine alterations were also investigated. While in proestrus carvacrol reduced serotonin and metabolite levels in both brain areas, no effects were observed in diestrus phase. Only in proestrus phase, carvacrol induced a depressive-like behavior in forced swimming test, without accompanying changes in ambulation. The improvement of performance in FST after subchronic treatment with fluoxetine (20 mg/kg) suggested a specific involvement of serotonergic system. No differences were found across the groups with regard to self-grooming behavior. Moreover, in proestrus phase, carvacrol reduced only estradiol levels without binding hypothalamic estradiol receptors. Our study showed an estrous-stage specific effect of carvacrol on depressive behaviors and endocrine parameters, involving serotonergic system. Given the wide carvacrol use not only as feed additive, but also as cosmetic essence and herbal remedy, our results suggest that an accurate investigation on the effects of its chronic exposure is warranted.

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Introduction

In addition to conventional medicine, complementary, alternative and herbal medicines are widely used across the world. In particular, the use of plant extracts is relatively high among women, especially among middle-aged and old women, with higher levels of education and higher incomes, and also with chronic diseases or poor overall health (Brett and Keenan, 2007). Although use of phytotherapy is prevalent in the general population, its use is even more common among women with depression (Eisenberg et al., 1993; Astin, 1998; Eisenberg et al., 1998; Unutzer et al., 2000; Kessler et al., 2001; Wang et al., 2001; Barnes et al., 2004).

At present, no data are available about the content of carvacrol in herbal remedies taken from women to automedicate. Many reports have documented evidence of the involvement of serotonergic system in the etiology of depression (Coppen et al., 1967; Stockmeier, 2003; Krishnan and Nestler, 2008) and although a dysfunctional serotonin system alone cannot explain the full pathophysiology, it is considered a key factor in depression (Michelsen et al., 2008). In this regard, tryptophan depletion studies confirmed the relationship between serotonin and this psychiatric disorder (Bell et al., 2001; Russo et al., 2009).

Oregano, thyme and majorana are native from the Mediterranean region east to eastern Asia. Several studies have reported that carvacrol (2-methyl-5-(1-methylethyl) phenol; C10H14O; mol. wt. 150.21) is the major natural constituent (70%) of essential oils of these aromatic plants (Baser, 2008) and the pharmacological actions of these essential oils are suggested to be parallel to carvacrol content (Aydin et al., 2007; Li et al., 2010).

Despite the large use of these herbal medicines, up to now little is known about the physiological effect of this terpene. Several studies underlined antimicrobial (Periago and Moezelaar, 2001; Kristinsson et al., 2005), antioxidant (Aydin et al., 2005; Horvathova et al., 2006) and anti-genotoxic in vitro activities (Ipek et al., 2005). Essential oils are also of potential interest as an alternative to antibiotic growth promoters, banned in the European Union because of safety issues

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Additionally, several evidences have demonstrated that plant-derived essential oils exhibit a variety of centrally active properties (Sousa et al., 2004; Silva et al., 2007). Indeed, due to their small molecular size and lipophilicity, volatile constituents of essential oils, such as carvacrol, are likely to readily cross the blood–brain barrier (Savelev et al., 2004). In particular, it has been suggested a possible modulatory action of carvacrol in the mammalian Central Nervous System (CNS) (Parnas et al., 2009). Besides, carvacrol seems to possess in vitro acetylcholinesterase inhibitory activity on the CNS (Jukic et al., 2007). However, to the best of our knowledge, no information is available on the effects of carvacrol on female reproductive physiology and endocrine profiles. Estrogen is a well-known regulator of mood, with reported effects of estradiol treatment ranging from depressant to antidepressant properties (Fink et al., 1998; Shors and Leuner, 2003). Furthermore, although many reports have linked estrogens to mood disorders in women, little is known about the role played by phytoestrogens and/or herbal medicines.

In the present study, by using in vivo and ex vivo approaches, we evaluated the neurochemical and behavioral profiles of adult female rats treated with carvacrol. Female rats were analyzed in two different phases of the estrous cycle, diestrus and proestrus, characterized by low and high levels of estrogens, respectively, in order to rule out the influence of gonadal hormones on the action of carvacrol. We evaluated possible alterations on monoaminergic transmission, which controls affective state and emotional responses. In particular, we investigated, 2 h after administration, serotonergic neurotransmission in rat prefrontal cortex (PFC) and nucleus accumbens (NAc) were affected by oral carvacrol treatment. Additionally, this study was designed to evaluate the effects of carvacrol in different behavioral tests to investigate for alterations in motor activity, depression and anxiety-related behaviors by using the open field and the forced swimming test. Finally, carvacrol-induced endocrine alterations were investigated in the plasma of female rats.

Methods

Animals. Adult female Wistar rats (Harlan, S. Pietro al Natisone, UD, Italy) weighing 200–250 g were used. The animals were randomly assigned to the experimental groups (n = 4–10 per group), one for each behavioral, neurochemical and biochemical analysis and they were allowed to acclimatize to the animal house for at least 7 days before the experiments. They were housed in standard cages in a controlled temperature room (22 ± 1 °C), and relative humidity (55 ± 5%) under a 12-h light/dark cycle (lights on from 8:00 AM to 8:00 PM). Standard laboratory chow and tap water were available ad libitum. The experiments were conducted in accordance with guidelines released by Italian Ministry of Health (D.L. 116/92 and D.L. 111/94–8), and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (NIH Publications No. 85–23, revised 1996). All efforts were made to minimize the number of animals used and their suffering.

Experimental protocol. After a week of habituation to housing conditions, female rats were subjected daily for two weeks to vaginal smears. Vaginal samples were taken every morning between 8:00 and 9:00 AM using a cotton stick inserted into the vagina not deeply. Then, vaginal fluid was placed on a glass slide and fixed with “Diff-Quick Staining Protocol”. It consists in fixing in “Diff-Quick” Fixative (Solution I) for 10 s, staining with “Diff Quick” (Solution II, pink) for 10 s and, finally, counterstaining with “Diff Quick” (Solution III, blue) for 8 s. Then, each slide was rinsed in water to remove excess stain and, when dry, observed under a light microscope, with 40× objective lenses.

The reproductive cycle of female rats is characterized by four phases: proestrus, estrus, diestrus and metestrus. The predominance of nucleated epithelial cells represented the proestrus phase, while the diestrus smear primarily consisted of a predominance of leukocytes with the presence of sporadic epithelial cells (Marcondes et al., 2002). Only regularly cycling rats (estrus cycle: 4–5 days) were assigned to experimental group, namely proestrus and diestrus, according to vaginal smear results obtained, on the test day.

Carvacrol (0.15 or 0.45 g/kg) or vehicle (peanut oil, 1 ml/kg) was orally administered in the morning immediately after vaginal cytology procedure. All experimental analyses were carried out before 2:00 PM, 2 h after carvacrol administration to avoid the influence of endocrine changes. Indeed during the estrous cycle, prolactin, LH and FSH remain low and increase in the afternoon of the proestrus phase (Marcondes et al., 2002).

Moreover, considering that the estrous cycle is very short, the association between carvacrol administration and changes in function was verified also at the end of each experiment, through the re-characterization of the vaginal smear.

Chemicals. Carvacrol (purity >98%), fluoxetine (20 mg/kg s.c. dissolved in deionized water) and unlabelled estradiol were purchased from Sigma Aldrich s.r.l. (Milan, Italy). The drug was dissolved in peanut oil and administered per os, by gavage, at two different doses (0.15 g/kg and 0.45 g/kg). Carvacrol and vehicle were administered in a volume of 1 ml/kg. Diff Quick Staining Solutions were purchased from Dade Behring (Milan, Italy). 3H-estradiol (Silva et al., 2007; 16,17-3H-(N)) estradiol) was purchased from PerkinElmer, Waltham, MA, USA.

Plasma sample collection. Trunk blood was collected from naïve or treated female rats in proestrus and diestrus phases using heparinised tubes. Samples were centrifuged at 10,000 x g for 20 min. at 4 °C. Supernatants were removed and frozen at −80 °C until analyses.

Progesterone quantification. Progesterone levels were measured by ELISA using commercially available kit (USCN Life Science Inc., Wuhan, China) according to manufacturer’s instructions. Briefly, colorimetric detection of peroxidase activity was achieved by adding TMB solution and incubating for 15 min at 37 °C according to the manufacturer’s instructions. The enzymatic reaction was stopped with Stop Solution and the optical density of each well was measured at 450 nm using a PowerWave XS plate reader (Bio-Tek, Winooski, VT, USA). Each analysis was performed in duplicate in the same assay to avoid inter-assay variations. Progesterone levels are expressed as ng/ml.

Estradiol quantification. Estradiol levels were measured by ELISA using commercially available kit (Cayman Chemical, Ann Arbor, USA) according to manufacturer’s instructions. The assay was based on the competition between estradiol and an estradiol–acyethylcholinesterase conjugate for estradiol antiserum. Optical density of each well was measured at 405 nm using a PowerWave XS plate reader (Bio-Tek, Winooski, VT, USA). Each analysis was performed in duplicate in the same assay to avoid inter-assay variations. Estradiol levels are expressed as pg/ml.

Post-mortem tissue analyses. Rats were killed by decapitation 2 h after drug administration and brains were immediately removed. For dissection, the brains were placed dorsal side up in an ice chilled rat brain matrix (World Precision Instruments, Inc. FL, USA) with slits spaced at 1 mm using an ice-chilled razor blade. Target regions were dissected out and weighted, according to the atlas of Paxinos and Watson (Paxinos and Watson, 1998). Thereafter, PFC and NAc were
collected and immediately frozen on dry ice. Tissues were stored frozen at −80 °C until serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) level quantification. At the time of analysis, samples were homogenized in 10 volumes (w/v) of 0.1 N perchloric acid. The homogenates were stored on ice for 30 min and then centrifuged at 10,000 ×g for 10 min at 4 °C. The supernatants were then filtered and diluted before high performance liquid chromatography (HPLC) analysis.

HPLC analysis. 5-HT and 5-HIAA concentrations were determined by HPLC coupled with an electrochemical detector (INTRO, Antec Leyden, The Netherlands). Separation was performed by a LC18 reversed phase cartridge column (hypersil, 150 mm×4.6 mm, ODS 5 μm; Thermoscientific, Milan, Italy). The detection was accomplished by a Unijet cell (BASI, Kenilworth, U.K.) with a 6 mm diameter glassy-carbon electrode at a working potential of 0.65 V vs. Ag/AgCl. The mobile phase used was 85 mM CH₃COONa, 0.8 mM octan sulfonic acid, 0.3 mM EDTA, 15 mM NaCl, methanol 6%, in distilled water, buffered at pH 4.85. The flow rate was maintained by an isocratic pump (Shimadzu LC-10 AD, Kyoto, Japan) at 1 ml/min. Data were acquired and integrated using Chromelon software (version 6.60, Dionex, San Donato Milanese, Italy). Substrate concentration was expressed as fmol per milligram of wet tissue.

Forced swimming test (FST). Rats were individually forced to swim inside vertical Plexiglas cylinders (diameter 23 cm; height 70 cm) containing 30 cm of water maintained at 25 °C. During a preconditioning period, animals were placed into the cylinders and, after 15 min in the water; they were removed and allowed to dry before being returned to their home cages. Twenty four hours later, each rat was returned to the water-filled cylinder for 5 min and recorded by video camera. During the test sessions, we measured the time that rats spent performing the following behaviors: struggling (time spent in tentative of escaping), swimming (time spent moving around the cylinder) and immobility (time spent remaining afloat making only the necessary movements to keep its head above the water).

Animals received subchronic treatment with fluoxetine (20 mg/kg s.c. dissolved in deionized water) 24, 5 and 1 h before FST.

Open field spontaneous locomotor activity. The apparatus consisted of a circular arena, 75-cm diameter, made of dark plastic under dim lighting. The experimental sessions were videotaped by a camera fixed above the arena. Animals were acclimated to the test room for 1 h before each test. Motor activity was measured by placing the rat into the center of the arena for 20 min session. The scoring was performed using a video-tracking motion analysis system (Ethovision, Noldus Information Technology, Wageningen, The Netherlands). To assess general locomotor activity, the following behavioral parameters (expressed as frequency on 5 min counts) were scored: number of square limit crossings with both forepaws, rearing (standing with the body inclined vertically, forequarters raised), and wall rearing (standing on the hindlimbs and touching the walls of the apparatus with the forelimbs).

To investigate anxiety-related behavior, we measured time spent performing general grooming activity consisting of: face grooming (strokes along the snout), head washing (semicircular movements over the top of the head and behind the ears) and body grooming (body fur licking) (Choleris et al., 2001).

Binding studies. All procedures were performed at ice/water temperature. Proestrus rat hypothalamic tissues, collected 2 h after carvacrol administration, were homogenized in 10 mM Tris–HCl containing 1.5 mM EDTA, pH 7.4 with a motor driven glass–glass Potter homogenizer 1.5 mL/g wet weight. Homogenates were centrifuged at 105,000 ×g for 60 min at 4 °C and the supernatants were used for receptor assays. Protein content was estimated by the Coomassie-blue method.

3H-estradiol saturation analysis. Increasing concentrations of 3H-estradiol ([2,4,6,7,16,17-3H-(N)] estradiol, specific activity 141 Ci/mmol, 0.625; 1.25; 2.5; 5; 10; 20; 40 nM) were incubated with proestrus rat hypothalamic cytosol (300 μg/ml protein) in polypropylene tubes in a 0.1 ml total volume, at 4 °C for 18 h for total binding. Nonspecific binding was estimated by another set of tubes containing 100-fold excess of unlabelled estradiol for each radioligand concentration. All assays were performed in duplicate. The reaction was terminated by adding 200 μl 0.5% dextran coated charcoal suspended in assay buffer. Then the unbound steroid was separated by pelleting through centrifugation at 1500 ×g for 15 min. 200 μl aliquots of the supernatants were transferred for scintillation counting into 4 ml Ultima Gold (PerkinElmer, MA, USA) and counted in a Tri-Carb (PerkinElmer, MA, USA) apparatus (64% efficiency).

Competitive binding experiments. These experiments were performed in quadruplicate and were carried out on proestrus rat hypothalamic cytosol using a constant concentration (5.0 nM) of radioligand (3H-estradiol) corresponding to a concentration approximately 2.5-fold greater than the estimated mean dissociation rate (Kd) in the absence or presence of increasing concentrations (0.5, 5 and 50 nM) of competitor (estradiol or carvacrol).

Statistical analysis. Results are expressed as means ± S.E.M. Statistical analyses were performed using Graph Pad 5.0 (GraphPad Software, San Diego, CA) for Windows. ELISA data were analyzed by Student’s t test. Neurotransmitter concentrations were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test. Behavioral data were analyzed by unpaired Student’s t-test. Scatchard analyses were performed for binding data. Differences were considered statistically significant when p value was less than 0.05.

Results

Sex hormone levels quantification

Characterization of the vaginal smear was performed according to Goldman et al. (2007). In order to confirm whether changes in vaginal epithelial cell structure were linked to alterations in circulating concentrations of the sex steroids, the endocrine pattern that underlies the changes in vaginal cell morphology was determined. In particular, estradiol levels were found to be significantly higher in proestrus (4.273 ± 0.41 pg/ml) compared to diestrus animals (2.267 ± 0.32 pg/ml). No difference was found in progesterone levels between proestrus (0.4730 ± 0.11 ng/ml) and diestrus (0.6694 ± 0.11 ng/ml) plasma samples of female rats.

Effects of carvacrol on serotonergic transmission in female rats during the proestrus and diestrous phases

In order to investigate if carvacrol possesses a neuromodulatory activity in female rat brains, and if gonadal hormones could influence the action of carvacrol, tissue levels of 5-HT and its metabolite, 5-HIAA, were measured in PFC and NAc during either the proestrus or the diestrous phase. Results showed that oral administration of carvacrol, at the highest dose (0.45 g/kg), caused a significant reduction in 5-HT and 5-HIAA content in PFC and NAc during the proestrus phase [PFC: 5-HT (F2,18 = 5.796 p < 0.05); 5-HIAA (F2,18 = 4.238 p < 0.05), Fig. 1a; NAc: 5-HT (F2,21 = 12.319 p < 0.001); 5-HIAA (F2,21 = 8.814 p < 0.01), Fig. 1b]. In diestrous period, oral carvacrol administration, at both doses tested (0.15 and 0.45 g/kg), had no effect on 5-HT and 5-HIAA concentrations in the PFC [(5-HT: F2,22 = 0.463 n.s.; 5-HIAA: F2,22 = 0.899 n.s.), Fig. 2a] and in the NAc [5-HT: (F2,24 = 0.888 n.s.); 5-HIAA: (F2,24 = 1.760 n.s.) Fig. 2b]. In both cycle periods, carvacrol did not alter the metabolism of 5-HT, given that no changes in 5-HIAA/5-HT ratio (an index of serotonergic
activity) were found in proestrus (Fig. 3a) and diestrus phase (Fig. 3b) in PFC (proestrus: F2,16 = 2.925 n.s and diestrus phase: F2,22 = 0.238 n.s, upper panel) and NAc (proestrus: F2,19 = 1.419 n.s; and diestrus: F2,24 = 0.964 n.s, lower panel) 2 h after carvacrol (0.15 and 0.45 g/kg p.o.) or vehicle (peanut oil, 1 ml/kg, p.o.) administration.

**Effects of carvacrol in FST**

To evaluate if neurochemical alterations had a functional significance, rats were subjected to the FST. Results showed that swimming time was significantly decreased (p < 0.01) and immobility time...
significantly increased (p<0.01) in carvacrol-treated female rats (0.45 g/kg) only in proestrus phase (Fig. 4a). No difference was found in immobility or swimming activity in diestrus phase (Fig. 4b). Climbing behavior was not affected in both experimental groups considered (Figs. 4a and b). For ethical reasons, to reduce the number of animals used, we chose to test only the dose of carvacrol (0.45 g/kg) able to evoke neurochemical alterations in our experimental conditions.

As represented in Fig. 4a, subchronic treatment with fluoxetine (20 mg/kg s.c.) was able to reverse the carvacrol effects on swimming (p<0.05) and immobility (p<0.01), thus confirming the involvement of serotonergic system in depressive-like action of carvacrol.

Effects of carvacrol in open field test

Carvacrol administration (0.15 and 0.45 g/kg p.o.) did not affect spontaneous locomotor activity in either diestrus or proestrus phase in female rats (data not shown). Similarly, no differences were found across the groups with regard to the self-grooming behavior (data not shown).

Effects of carvacrol on estrogen receptors

To verify if carvacrol exerted its action by directly interacting with estrogen receptors, we performed a binding study in hypothalamic cytosolic fractions from proestrus rats. This area was chosen because it is particularly rich in estrogen receptors (Pfaff and Keiner, 1973). As shown in Fig. 5, carvacrol was not able to displace hypothalamic estradiol binding. On the contrary, our positive control, unlabeled estradiol, dose-dependently competed with 3H-estradiol.

Effects of carvacrol on gonadal hormones

Fig. 6 clearly shows that oral administration of carvacrol (0.45 g/kg) significantly reduced plasmatic estradiol levels only during the proestrus (Student’s t test, p<0.001), while no effects were observed during the diestrus phase. Moreover, oral carvacrol administration did not modify plasma progesterone levels in both phases of estrous cycle.
Discussion

The present study suggests that carvacrol-treated female rats show a depressive-like phenotype, depending on ovarian cyclicity. Female rats were analyzed in two different phases of the estrous cycle, diestrus and proestrus, characterized by low and high levels of estrogen, respectively, in order to consider the influence of gonadal hormones on the effects of carvacrol.

Interestingly, our data showed that carvacrol has an estrous-stage specific effect on depressive behaviors and endocrine parameters. Indeed, we have demonstrated that the acute administration of carvacrol significantly increased immobility time during the FST in female rats, only during the proestrus phase. These changes were prevented by subchronic treatment with the antidepressant fluoxetine.

The original view of the FST offered by Porsolt was based on a model of depressive state mimicked by learned helplessness (Porsolt et al., 1977). This test allows analyzing active behaviors aimed to escape from stressor condition, such as struggling or swimming and passive behaviors, resembling a state of despair, such as immobility.

The immobility behavior displayed in rats when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair and has been suggested as an animal model of human depression (Porsolt et al., 1977; Bach-Rojecky et al., 2004).

To avoid false positive/negative results in the FST, it is important to rule out the possibility that alterations in immobility time are not merely due to an alteration of general motor behavior. In our study, carvacrol, at the same dose that produced a depressant-like phenotype, did not alter locomotor activity compared to control group in the open field, a classical animal test used to evaluate spontaneous motor activity. Therefore, it is conceivable to suggest that the effects observed in the FST during the proestrus phase could not be attributed to a general reduction in locomotor activity. Nevertheless a possible anxiogenic effect of carvacrol is doubtful, because no differences were revealed in time spent in grooming activity, an index of increased anxiety in the open field test (Britton and Britton, 1981; Choleris et al., 2001) between carvacrol-treated female rats in proestrus phase with respect to the control animals. Therefore a specific effect of this compound on the behavioral paradigm could be hypothesized, that is suggestive of depression.

In particular, carvacrol-treated rats showed an increase in immobility and a decrease in swimming behavior in the FST whereas struggling was unchanged. The depressive-like effects induced by carvacrol were reversed by subchronic treatment with fluoxetine. Literature data demonstrated that selective 5-HT reuptake inhibitors, such as fluoxetine, selectively reduced immobility and increased swimming, but not struggling, behavior, whereas selective noradrenaline reuptake inhibitors and tricyclic antidepressants preferentially decreased immobility, and increased struggling, but not swimming behavior (Detke et al., 1995; Cryan et al., 2005). Accordingly, we have assumed that the mechanism through which carvacrol induced this effect could be an alteration in 5-HT levels. To confirm this hypothesis, neurochemical analyses were conducted. Results showed that in carvacrol-treated animals, 5-HT and 5-HIAA levels were reduced in both PFC and NAc, two specific cerebral areas belonging to the emotional circuit. In addition, 5-HT turnover, evaluated by the ratio of 5-HIAA against 5-HT, did not differ significantly between control and treated rats.

Many reports have documented evidence of the involvement of serotonergic system in the etiology of depression (Coppen et al., 1967; Stockmeier, 2003; Krishnan and Nestler, 2008) and although a dysfunctional serotonin system alone cannot explain the full pathophysiology, it is considered a key factor in depression (Michelsen et al., 2008).

Impaired serotonergic transmission in the PFC is central to depressive disorder and there is accumulating evidence that the NAc plays an important role in the pathophysiology of depression (Parsons et al., 1995; Krishnan and Nestler, 2008; Liang et al., 2008).

Interestingly, carvacrol selectively reduced 5-HT content in female rats in proestrus phase but not during the diestrus period confirming the estrous-stage specific effect of carvacrol. Actually, alteration in the functioning of this system is largely influenced by several endogenous factors such as sexual hormones. Gonadal hormones affect the nervous system in ways that extend beyond their essential actions of modulating sexual behavior. A growing body of literature describes the effects of estrogens on the CNS. Estrogens effects upon the serotonergic system may contribute to sex differences in brain function and also include gender differences in the incidence of psychopathologies such as depressive illness, which is more common in women (Zhou et al., 2002; Darnall and Suarez, 2009).

It is worthy to note that, during the FST, estradiol benzoate-treated ovariectomized animals spent significantly less time in immobility and spent most of the time in swimming (Rachman et al., 1998). Moreover, in ovariectomized rats, estradiol induced an increase in the density of 5HT2A receptor in many regions of forebrain involved in the control of mood (Summer and Fink, 1995) and, in intact rats, the density of 5HT2A receptors was shown to be increased in PFC and NAc at proestrus (Summer, 1997). Estradiol seemed to turn on 5-HT2A receptor gene transcription in the dorsal raphe to increase receptor density in cortical serotonergic projection areas (PFC and NAc) (Summer and Fink, 1998).

Based on the data reported above, our results support a view in which carvacrol, during the proestrus phase and by reducing 5-HT content, seems to promote the passive behavioral pattern in an estrous-phase dependent manner. Therefore, the present results raised the hypothesis that carvacrol effects on FST and serotonergic system are dependent on estrogen levels.

To assess directly whether carvacrol induces endocrine alterations, as the above data suggest, we measured circulating sex hormone concentrations. Interestingly, we found that acute administration of carvacrol was effective in significantly reducing plasma estradiol levels only during the proestrus phase. Progesterone levels were not modified. Moreover, no effects were observed on the interaction between carvacrol and estrogen receptors, since displacing binding
study failed to reveal a direct interaction, at least in our experimental conditions. To the best of our knowledge, this is the first report showing that carvacrol, at the higher dose used in this study, might have pro-depressant properties in female rats. Together with the reduction of plasma estradiol concentrations in proestrus phase, this natural constituent of essential oils selectively reduced 5-HT content in the PFC and NAc inducing, in spontaneously cycling female rats, a state of despair which is reversed by a selective inhibition of 5-HT reuptake.

In conclusion, the findings described here provide novel outcomes that make a contribution in the research on the effects of phytochemicals on CNS. Taking into consideration its lipophilic profile, carvacrol, which is abundant in several aromatic plants, easily and rapidly cross the blood–brain barrier and it should be cautiously considered that might accumulate in adipose tissue. Then, given the wide carvacrol use not only as feed additive, but also as cosmetic essence and herbal remedy, our results suggest that an accurate investigation on the effects of its chronic exposure is warranted.

Conflict of interest

The authors declare no conflict of interest.

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