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Administration of curcumin affects the anxiety behavior in Wistar rats after formaldehyde injected

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Abstract The curcumin is part of the curcuminoids family. Present in the spice turmeric is a vegetable alkaloid obtained from *Curcuma longa*. Many studies have been carried out to describe the multiple biological actions of curcumin. The positive antidepressant and anti-anxiety effects of curcumin are most likely due to its ability to normalize specific physiological mechanisms, in addition to being a potent antioxidant and anti-inflammatory agent. Curcumin also protects the brain and simultaneously acts on several mechanisms related to depression. It is considered to be the most promising therapeutic target for treating depressive behaviors. Through this work, we will prove the protective effect of curcumin on the anxious behavior of Wistar rats and the complications induced by the administration of a toxic product that is formaldehyde. To this fact, 25 white rats of Wistar strains (250±50 g) were separated into five experimental batches: control batch (T; n = 5), vehicle control batch (CV; n = 5), Curcumin control batch (CC; n = 5), one batch formaldehyde vehicle (FV; n = 5) and one batch formaldehyde Curcumin (FC; n = 5). A single intraperitoneal injection carried out the formaldehyde administration diluted in distilled water at a dose of 10 mg/kg. Turmeric dissolved in olive oil was administered by gastric gavage at a dose of (60 mg/kg). Our results show the therapeutic benefits of curcumin against the deficits caused by formaldehyde reflected in a disturbance in rat behavior and degree of anxiety using the open-field test and the light and dark box test.

Keywords animal behavior, anxiety, *Curcuma longa*, open-field test, tumeric

1. Introduction

Curcumin (CUR) is a frequent spice used in the diets of Asians, like India or China. Its antioxidant and anti-inflammatory properties have been exploited for centuries for the treatment of several chronic diseases. Besides, its usefulness as a complementary treatment in several neuropsychiatric disorders has been reported by several authors; the number of trials testing its effectiveness in mental health conditions has steadily increased over the years, both in animal models and in humans. Because of these properties, it has been speculated that curcumin may help treat depression, affecting millions worldwide (World Health Organization 2017).

CUR has been used for centuries in medicines to treat a variety of inflammatory symptoms. It is a commonly used spice as it is increasingly studied for its antidepressant effect. (Lopresti and Drummond 2017). CUR has also shown anxiolytic-like effects on the biochemistry and behavioral symptoms associated with anxiety (Lee and Lee 2018).

Anxiety is a common and potentially serious condition that can significantly affect individuals' quality of life and predispose them to psychiatric comorbidities. Depression is a consequence of anxiety, affecting 21% of populations (Esmaily et al 2015).

Epidemiological studies show that people complain of a series of neuropsychiatric symptoms, such as depression, anxiety, sleep disturbances, malaise, balance dysfunctions, headaches, indigestion, lethargy, decreased motor activity, and loss of appetite. These further confirmed that neuropsychiatry symptoms are closely related to long-term exposure to formaldehyde in the air (Wang et al 2017).

The formaldehyde, better known as formalin when dissolved in water, is a highly volatile organic compound belonging to the aldehyde family. Low molecular weight, this substance has the property of becoming gaseous at room temperature and is currently frequently found in indoor environments (Erika 2015).

Formaldehyde is known to have these deleterious effects on human health (Lan et al 2015). The commercially available formaldehyde contains 37-41% formaldehyde, classified as carcinogenic to humans (Hoque 2018).

In this context, our study aims to assess the protective effect of the natural molecule (curcumin) against the harmful effects induced by formaldehyde through behavioral tests aiming to verify the rats' possible presence of central nervous functions.

2. Materials and Methods

2.1. Biological material

The experimental study was carried out on 25 white rats of the Wistar strain, weighing on average 150g on their arrival (250±50 grams). These animals were acclimatized to animal housing conditions at a temperature of 25±2 °C; the animals were reared in polyethylene cages lined with litter made from wood chips. The cages were cleaned, and the litter changed once every two days. Feed brought to the

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animals was made in the form of sticks made of corn, barley, milk, and vitamin supplements. Drinking water was presented in bottles adapted to the cages. Food and water are provided *ad libitum*. After an adaptation period of 4weeks, the rats were separated into five experimental groups: control batch (T; n = 5), vehicle control batch (CV; n = 5), Curcumin control batch (CC; n = 5), one batch formaldehyde vehicle (FV; n = 5) and one batch formaldehyde Curcumin (FC; n = 5). The whole approach described is in agreement with the University of Badji Mokhtar's ethics guidelines–Annaba.

2.2. The light/dark box test

The light/dark box test is based on Crawley and Goodwin's original model (1980). This test encountered the conflict between rodents' natural urge to explore their fear of light. They naturally prefer dark places while avoiding bright places (Ben Ahmed 2016). The test was carried out in a box divided into two compartments, one compartment painted in black and the other in white illuminated by daylight. There is an opening that serves as a door that has been created between the two zones to facilitate the easy movement of the animal from one region to another. First, the rat was placed in the lighted compartment at this time and start filming the test for 5 minutes in two sessions, on day 7 and day 14, using a high-performance camera.

2.3. Test of the open-field (OF)

The test was described by Hall (1934) to measure differences in emotional reactivity. The device consists of a square base surrounded by plexiglass walls with 70×70×40 cm. The test was based on the rat's delicate placement, picking it up by the field's center queue. A high-capacity camera filmed the evolution of its behavior for 5 minutes in 2 sessions on day 7 and day 14. At the end of each test, the rat was removed and clean entirely with a 70% alcohol solution and dry with absorbent paper. Its movement makes it possible to measure the time spent in the peripheral zone and the central zone. Therefore, this test indicates the anxious behavior of the rats.

2.4. Administration of formaldehyde

The administration of formaldehyde solution meets the analytical specification of USP; ≥34.5 wt. % was carried out by a single intraperitoneal injection at a dose of 10 mg/kg according to Mahmoud and Fenghour (2016) after dilution with distilled water.

2.5. Administration of antioxidants

The curcumin used for the treatment of rats was presented as a yellow powder. Turmeric (*Curcuma longa L.*) was obtained in our research laboratory to prepare an adequate dose. A precision balance was used to measure a dose of 60 mg/kg depending on the rats' weight. The quantified doses were added to Eppendorf tubes to be dissolved in olive oil. Before administration, the tubes were shaken well until a homogeneous solution was obtained. The treatment began on the 8th day after the formaldehyde injection, and its administration was by gastric tube for seven days.

2.6. Data processing

The data were introduced then processed with the Prism software (version 6). The results are expressed as mean±SEM and compared by a student's *t*-test at the significance level Alpha = 0.05 then represented in histograms.

3. Results

3.1. Variation of the light/dark box test parameters

Figure 1 shows the rats' time in each box compartment, light and dark, in the two sessions on day 7 and day 14. On day 7, the batches FV and FC spend more time in the dark compartments with a very significant difference ($P < 0.01$) compared to batch T. On day 14, a non-significance ($P > 0.05$) between FV and FC was observed. On the other hand, a significant difference ($P < 0.05$) was noticed between FV day 7 and FV day 14, with averages of 253.0±6.95 s and 229.8±10.36 s. Another very significant difference ($P < 0.01$) between FC day 7 and FC day 14 with a mean of 252.2±5.21 s and 211.0±6.96 s, respectively.

On day 7, a very significant decrease in the time spent in the clear compartment of the FV (47.00±6.950 s) and FC (47.80±5.21 s) compared to T (77.80±3.84 s). On day 14, a non-significance difference between FV (70.20±10.36 s) and FC (89.00±6.96 s), compared to T (82.60±7.76 s), was observed. There is a significant difference between FV (47.00±6.95 s) day 7 and FV (70.20±0.36 s) day 14. Also, a very significant difference between FC (47.80±5.21 s) day 7 and FC (89.00±6.957 s) day 14 was verified.

3.2. Variation of the open-field test parameters

Figure 2 reveals the time spent in the two zones of the open-field test's specific device in two sessions' day 7 and day 14. The time spent in the peripheral zone, on day 7, was a highly significant increase ($P < 0.01$) of the FV and FC (Figure 2A). On day 14, there is no significant difference between the lots, although a highly significant difference ($P < 0.01$) between the two sessions of the same batch FV (day 7 and day 14) was observed. For the batch treated with curcumin (FC), a very highly significant decrease ($P < 0.001$) was verified between day 14 (229.4±6.92 s) and day 7 (283.0±5.0 s).

Figure 2B shows the time spent in the central zone and the results contrast with those of the peripheral zone. We notice a highly significant difference (P < 0.01) between FV (18.80±3.65 s) and FC (17.00±5.03 s) when these are compared with T (60.00±11.32 s). On day 14, an absence of significance between the batches was observed. A very highly significant increase ($P < 0.001$) of the batch FC on day 14 (70.60±6.92 s), when compared to the FC day 7 (17.00±5.03 s), was observed.

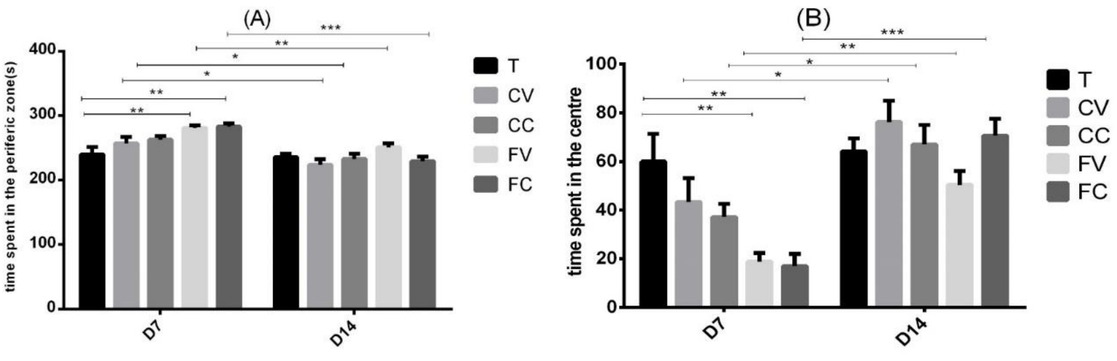

Figure 1 Time spent in the light/dark box of the batches: controls (T), vehicle control (CV), curcumin control (CC), vehicle formaldehyde (FV), and curcumin formaldehyde (FC). The results are expressed on mean±SEM. Ns. is non-significant difference = $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

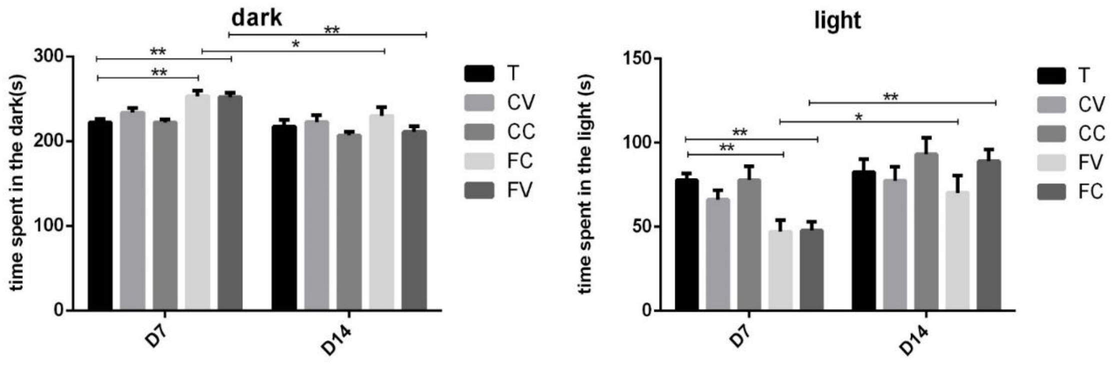

Figure 2 Time spent in the peripheral zone and the central zone of the open field of the batches: controls (T), vehicle control (CV), Curcumin control (CC), vehicle formaldehyde (FV), and curcumin formaldehyde (FC) the results are expressed as mean±SEM. Ns. is non-significant difference = $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

4. Discussion

Our research has based on two parameters of the light/dark box test: the time spent in the light compartment and the time spent in the dark chamber. We recorded on the 7th day of the rats which were injected with formaldehyde (FV) and (FC) spend more time in the dark compartment than the clear chamber, with a very significant difference compared to the control (T). It is known that mice naturally prefer dark places and avoid lighted ones. This natural behavior has been used to estimate the degree of anxiety in animals (Arrant 2013). Our results explain that formaldehyde acted on rats' behavior by inducing an increase in anxiety level. This is in agreement with work that demonstrated behavioral sensitization produced after injection of formaldehyde in mice for 7 days, suggesting anxiety caused by formaldehyde (Li 2016); nevertheless, it had no batch significance (FV) and (FC) of day14.

On the other hand, when the identical batches from the two sessions were compared, the rats treated with the vehicle (FV) J7 and (FV) J14 also which were treated with curcumin (FC) day 7 with (FC) day 14.

We did notice an improvement in behavior at day 14 precisely in the batch that we treated with curcumin that presented itself with a very significant decrease at day 14 in the dark zone this

The results were consistent with the very significant increase in time spent in the clear zone. Behavioral tests were performed in epileptic rats with epilepsy induced by an intraperitoneal injection of a single dose of kainic acid and subsequently treated with curcumin. The epileptic rats treated with curcumin exhibited anxiolytic behaviors in the light/dark box test, which is less observed in rats treated with the vehicle (Yow et al 2017). Other research has measured certain behaviors related to anxiety—responses to examine the effects of curcumin on rats' anxiety symptoms (Lee and Lee 2018). Besides, turmeric also significantly reverses the cognitive and behavioral change in rats. (Huang et al 2011; Morrone et al 2016; Choi et al 2017).

The open-field test was used to evaluate the locomotion and rats' exploratory behavior. In this context, we chose two parameters to study, which are presented by the time spent in the peripheral zone and the central zone of this test's specific device. On day7, it was observed that the rats injected with formaldehyde spent more time in the peripheral zone and little time in the central zones. This explains why formaldehyde played the role of a stressful and exciting toxic product. Exposure to different FA concentrations in mice affects locomotor activity, depressive and anxious behavior, and cognition like other chemical aggressors (Li et al 2016). Thus, exposure of 2 ppm of FA in rats for a week significantly reduced locomotor activity in the open-field (Hoque et al 2018).

On day 14, an improvement was certainly noticed in the batch treated with curcumin with a very highly significant increase between the (FC) day 14 and the (FC) day 7 of the time spent in the central zone, unlike the peripheral zone. Administer CUR to rats after involving prolonged stress by reversing serotonin (5-HT) dysfunction. Demonstrated a significant decrease in anxiety behaviors, as indicated by an increase in the number of central zone crossings during the open-field test. (Usanmaz et al 2002).

5. Conclusions

In this work, we demonstrated that curcumin's oral administration, one of the spices used as a natural treatment in Wistar rats, reveals preventive efficacy against anxiety induced by single intraperitoneal injection formaldehyde. We used two behavioral tests with these results to prove our expectations: the open-field test and the light and dark box.

Conflict of Interest

The authors declare that there are no conflict of interest with this work.

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