OPEN ACCESS
Journal of Animal Health and Production

Research Article

# Evaluation of Acute Toxicity, Antidepressant, Antioxidant Activities and Memory Ability of *Curcuma longa* Methanolic Extract in High fructose-treated rats

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**Abstract** | *Curcuma longa* is a rhizomatous herbaceous plant used as a spice and food supplement, known for its valuable natural bioactive compounds. This study aims to investigate the acute toxicity, antidepressant effects, antioxidant activities, and memory enhancement potential of methanolic extract of *Curcuma longa* (MECL) in high fructose-treated rats. To assess acute toxicity, rats were divided into five groups treated with different doses of MECL (2000, 3000, and 4000 mg/kg) for 14 days. For the evaluation of antidepressant, antioxidant activities and memory performance, rats were randomly distributed into six groups included Control, Group (2) provided with a 23% fructose solution for one month; Group (3), administered 0.1 g/kg/d of MECL for 10 days, followed by fructose solution; Group (4) treated with 30 mg/kg/d of losartan for one month alongside a fructose solution; while Group (5), subjected to a combination of MECL, losartan, and a fructose-based diet, and finally a Group (6), receiving MECL and losartan supplementation. The MECL used contain 48.70% β-turmerone and 10.64% α-turmerone. For the 2000mg/kg group, no variation in clinical signs of toxicity was observed during the acute toxicity test. One month of fructose consumption led to the development of depressive and anxious behaviors as well as memory deficits, as revealed in various behavioral tests, including forced swimming test, open field test, elevated plus maze, Y-maze test and Morris water maze. Furthermore, the supplementation with MECL effectively mitigated the depressive behaviors and prevented the memory impairments induced by fructose consumption. Thus, the present study supports the use of *Curcuma longa* as a potential nutri-therapeutic agent in mood regulation and memory enhancement.

**Keywords** | Curcuma longa, Losartan, Behavior, Memory, Obesity, Toxicity.

**Received** | December 17, 2023; **Accepted** | April 06, 2024; **Published** | May 10, 2024
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**Citation** | Brikat S, Kherrab I, Taaifi Y, Haida S, Ibouzine-Dine L, Abouyala O, Fitah I, Mesfioui A, Elhessni A (2024). Evaluation of acute toxicity, antidepressant, antioxidant activities and memory ability of *Curcuma longa* methanolic extract in high fructose-treated rats. J. Anim. Health Prod. 12(2): 212-223.
**DOI** | http://dx.doi.org/10.17582/journal.jahp/2024/12.2.212.223
**ISSN** | 2308-2801



## INTRODUCTION

The use of natural origin substances is an innovative spring often underestimated by the healthcare sector. According to the World Health Organization (WHO), plants provide healthcare to 80% of the world's population, and the demand for these services is on the rise. The WHO, through its strategy for traditional medicine for 2014-2023, strongly advocates for traditional medicine grounded in scientific evidence, with a focus on ensuring

the quality, safety, and efficacy of these natural products. The potential benefits of natural therapeutic products are substantial; however, associated risks, including direct and negative side effects and undesirable therapeutic interactions have to be taken into consideration. Thus, encouraging investment in research is a necessity to harness the advantages of these accessible and affordable natural therapies. Such research holds the promise of developing new treatments and advancing effective and safe therapies. As an example, obesity has significantly increased in recent decades representing a risk for the development of cardiovascular and metabolic diseases, as well as neuropsychiatric conditions which can be accompanied with high incidence of depression, anxiety, and cognitive disorders (Kherrab et al., 2024a). Addressing these disorders is, therefore, crucial in the battle against obesity (Fourrier et al., 2016). There exist complex associations of neurobiological mechanisms responsible for the emergence of neuropsychiatric disorders linked to obesity. Numerous studies have shed light on the role of inflammation in this process. In fact, obesity is closely interlinked with an inflammatory response, underscoring the dynamic interplay between fat cells and the immune system (Fève et al., 2006). As a result, there is an upsurge in the circulation of pro-inflammatory factors, including cytokines, which surpass the levels observed in non-obese individuals. These released cytokines can apply their influence on various organs, including the brain. They trigger the production of cerebral cytokines by glial cells, key players in regulating mood and cognition. These cytokines also impact metabolism, the function of specific neurotransmitters, the activity of the hypothalamic-pituitary-adrenal axis, and neuronal plasticity (Fourrier et al., 2016). This intricate interplay within our bodies underlines the effects of obesity on both our physical and mental health. Many plants have been employed to treat obesity, with *Curcuma longa*, a rhizomatous herbaceous plant renowned for its role as a spice and versatile food supplement, standing out for its significant medicinal and nutritional value. Several studies have showed that *Curcuma longa*, revealing its multifaceted effects, including anti-inflammatory and anthelmintic effects (Khattak et al., 2005), neuroprotective qualities, and anti-amyloid and antioxidant activities (Askarizadeh et al., 2020). Moreover, this remarkable plant has been linked to enhancing spatial memory by reducing chronic neuroinflammation and promoting hippocampal neurogenesis (Brikat S et al., 2024). It achieves this through the activation of BDNF/Trkb/PI3K/Akt signaling (Sun et al., 2020). In fact, it has been demonstrated that long-term treatment with *Curcuma longa* has resulted in beneficial effects on learning and memory functions in rats (Su et al., 2010). In light of these promising findings, the primary focus of the present study is to delve and evaluate the acute toxicity, antidepressant, antioxidant activities and memory performance of *Curcuma longa*.

## MATERIAL AND METHODS

### Preparation of *Curcuma Longa* Methanolic Extract

The Curcuma rhizomes were first dried at 105°C for 3 hours, then triturated and sieved to obtain a uniform powder with a size of 0.18 mm. The powder was then stored in a refrigerator following the method described by Sahne et al. (2016). Subsequently, 150 grams of this *Curcuma longa* powder was extracted using 80% methanol in a Soxhlet extractor, maintained at 60°C for a duration of 4 hours. The solution was then filtered to remove the solvent, and the separated solution was processed using a rotary evaporator under vacuum conditions at 60°C for 30 minutes, rotating at 2000 revolutions per minute (rpm).

### Gas Chromatography-Mass Spectrometry Analysis

For the Gas Chromatography-Mass Spectrometry Analysis (GC-MS) analysis, 1 ml of the Methanolic Extract of *Curcuma Longa* (MECL) was subjected to analysis using the GC-MS liquid chromatography technique, specifically employing the Focus-ISQ system by Thermo Scientific. The separation of compounds was achieved using a ZB-5ms capillary column (30 mx 0.25 mm Φ, Phenomenex) and a temperature program ranging from 40°C to 300°C for 2 minutes. The extract compounds were then identified through Kova indices, known in the standards and literatures of Adams (2004) and Babushok et al. (2011).

### Diphenylpicrylhydrazyl Free Radical Scavenging Test

In the Diphenylpicrylhydrazyl (DPPH) free radical scavenging test, we followed the protocol described by Haida and Kribii in 2020. The solutions of the extracts were diluted to various final concentrations, ranging from 0 to 0.5 mg/ml. Subsequently, 2 ml of a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution at a concentration of 76 mM was prepared in ethanol and added to 0.1 mL of the extract solution. This mixture was incubated, and after a 30-minute incubation period, the absorbance was measured at 517 nm using a spectrophotometer (UV-2005, Selecta). The results were then converted to a percentage of inhibition of the DPPH radical, calculated using the following formula: Inhibition (%) = [(Ablank - Asample) / Ablank] x 100. The Half maximal inhibitory concentration (IC50) value, which represents the sample concentration required to inhibit 50% of the free radical DPPH, was determined using the regression line equation, focusing on the linear part of the graph.

### Acute Toxicity Experiment

The experimental acute toxicity test was performed on 30 adult male Wistar rats, each approximately 3 months old.

The selection of these rats was made randomly, in accordance with the guidelines by the NIH Guide for the Care and Use of Laboratory Animals. These rats were sourced from the local breeding colony within the Laboratory of Biology and Health, Department of Life Sciences, located at the Faculty of Sciences, Ibn Tofail University. Prior to the experiment, the rats were acclimatized in a well-lit environment for 12 hours a day, maintained at a constant temperature of 22°C, and provided with unrestricted access to food and water for a period of 5 days. In their housing, six rats were accommodated per cage. On day prior to the experiment, the rats were subjected to an overnight fasting period. The rats were then categorized into five distinct groups, each comprising 6 rats (n=6), as detailed in Table 1. To ensure ethical treatment, all experimental procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

**Table 1:** Acute Toxicity Experiment Groups

| Group | Treatment |
|---|---|
| Group1 | Physiological saline |
| Group 2 | DMSO |
| Group 3 | 2000 mg/kg of MECL |
| Group 4 | 3000 mg/kg of MECL |
| Group 5 | 4000 mg/kg of MECL |

DMSO. Dimethyl sulfoxide; MECL. methanolic *Curcuma longa* extract

## Acute Toxicity Test

In the acute toxicity test, Methanolic Extract of *Curcuma Longa* (MECL) was administered to rats at doses of 2000, 3000, and 4000 mg/kg of body weight. This experiment aimed to establish both the No-Observable-Effect-Level (NOEL) and the Lethal Dose (LD50), which represents the dose resulting the death of 50% of the tested animals. The rats were subjected to these doses for a duration of 14 days, during which various physiological parameters were closely monitored.

## Mortality and Clinical Signs

Throughout the course of the experiment, a thorough daily monitoring of the rats was conducted to observe clinical signs, which encompassed behavioral changes, posture, scratches, fur appearance, aggressiveness, paralysis, sedation, drowsiness, tremors, and asphyxiation. Additionally, mortality rates were also monitored keeping track of any fatalities among the subjects.

## Biochemical Parameters

A comprehensive biochemical study was carried out to assess the effects on certain key biochemical parameters within the animals' blood, focusing on glucose and HDL cholesterol levels. On the 14th day of the experiment, six rats were randomly selected from each group, and they were subsequently anesthetized. Blood samples were collected and analyzed by examining the blood serum after centrifugation at 3000 revolutions per minute (r/min) for a duration of 10 minutes (Kherrab et al., 2024b).

## Weight-related Organs

In the final phase of the experiment, selected subjects underwent a weight assessment, after which they were humanely sacrificed. The organs, including the liver, kidney, heart, and brain, were then carefully removed and weighed.

## High fructose-treated Animals

We utilized a total of thirty-six one-month-old "Wistar" female rats, each with an initial weight of 75 ± 10 g. These rats were sourced from the local breeding colony within the Laboratory of Biology and Health, situated in the Department of Life Sciences at the Faculty of Sciences, Ibn Tofail University. The rats were subjected to a hypercaloric diet with high fructose content (23% w/v). Throughout the experiment, they had unrestricted access to this diet for a duration of one month. The environmental conditions were maintained with a natural photoperiod, consisting of 12 hours of light and 12 hours of darkness, as well as a standard temperature of 22°C. The weight of each rat was measured at the end of every week (Lindqvist et al., 2008). This comprehensive approach ensured that the experimental procedures were conducted under controlled and standardized conditions.

## High fructose-treatment Experimental Design

A total of 36 female rats were randomly distributed into six groups, each comprising six individuals (n=6). The experimental design encompassed the following groups: the Control Group with standard chow and water access, Group F with a 23% fructose drinking solution for one month, Group C+F combining Methanolic Extract of *Curcuma longa* (MECL) at 0.1g/kg/d for ten days via force-feeding with fructose solution, Group L+F administered losartan at 30mg/kg/d for one month followed by fructose solution, Group C+L+F receiving MECL, losartan, and a fructose diet, and Group C+L treated with MECL and losartan but excluding the fructose diet. The study spanned 115 days since the birth of the female rats (Figure 1) (Brikat et al.,, 2023).

## Behavioral Assessment

**Open Field Test:** The Open Field Test (OF) is a method used to evaluate anxiety levels caused by exposure to well-lit spaces in rodents (Tariq et al., 2020; Bassani et al., 2017; Oyemitan et al., 2017). This test was built using white plywood and measured 100 x 100 cm, surrounded by 40 cm high walls. The open field was divided into 25 squares, organized as 9 central squares and 16 peripheral squares

(Ramos et al., 1997). Individual animals were placed in the center of the device and allowed to freely explore their surroundings for a duration of 5 minutes. The recorded parameters included the number of central squares visited and the time spent within these central cells, offering insights into the animals' exploratory behavior and anxiety levels.

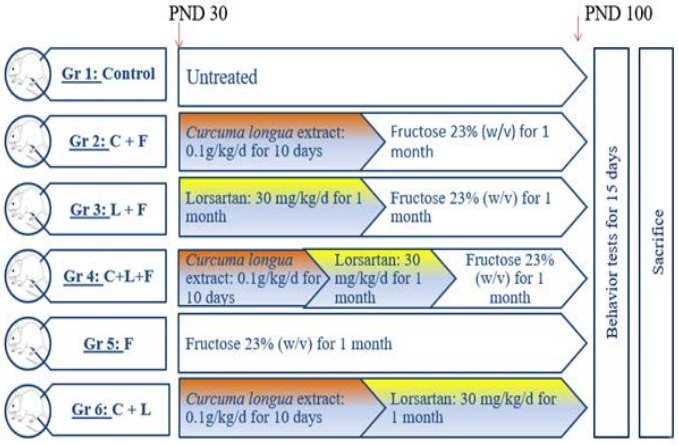


**Figure 1:** Experiment design of high fructose-treatment on 6 groups of female rats. C. *Curcuma longa*, F. fructose, L. losartan, PND. Postnatal day.

**Elevated Plus Maze Test:** The Elevated Plus Maze test (EPM) is employed to evaluate anxiety-like behaviors in rats, as demonstrated in studies by Tariq et al. (2020), Bassani et al. (2017), Oyemitan et al. (2017), and Da Silva Morrone et al. (2016). The maze itself consists of a cross-platform elevated one meter above the ground with two enclosed arms measuring 50 × 10 × 40 cm and two open arms measuring 50 × 10 cm. When conducting the test, rats were placed outside the maze, facing one of the open arms, with the open arms and the elevated labyrinth generating anxiety in the subjects. After each test, the device was meticulously cleaned with ethanol to eliminate any traces from previous animal subjects, in accordance with the method detailed by Garcia et al. (2004). The time spent in the open arms and the number of entries into the open arms were both measured and served as reliable indicators of anxiety-like behaviors (Ramos et al., 1997). An entry is counted when all four legs of the rat are positioned on the arm.

**Forced Swimming Test:** The Forced Swimming Test (FST) is used to examine depressive-like behavior in rodents (Porsolt et al., 1977). It involves a cylindrical container with a height of 50 cm and a diameter of 30 cm, filled with water to a depth of 27 cm, maintained at a temperature of 22°C. During the test, individual animals were placed into the water for a duration of 5 minutes. The swimming sessions were recorded using a video camera positioned directly above the cylinder, with the absence of experimenters during the recording to minimize interference. After each test, the water in the cylinder was replaced. The primary parameter measured in the FST was the immobility time, which expresses the duration the rat spends in a state of immobility floating in the water and making only the necessary movements to maintain the nostrils above the water (Wang et al., 2007).

## Memory Assessment

**Object Recognition Test:** The test Object Recognition Test (OR) assesses the short-term memory of rodents and involves a cubic test box measuring 40 $cm^3$. On the first day, rats were acclimatized for a period of 5 minutes. The test itself consisted of two parts. In the first part, referred to as the acquisition phase and conducted on the second day, two objects of the same height and consistent width with the same color were placed in the test box. The rats were positioned in the center of the box and allowed a 5-minute exploration period to become familiar with the objects. Twenty-four hours later, one of the two previously encountered objects was replaced with a new object of a different color (the new object) and presented to the same rats for a 5-minute exploration period. Various parameters were measured during this test, with a primary focus on the recognition index. The recognition index corresponds the proportion of time the animal spends on the new object and ranges from 0% to 100% (Bevins & Besheer, 2006).

**Y-Maze Test:** The Y-Maze test is employed to study a rat's natural tendency to make alternate choices after exploring a branch of the maze. This test is particularly used to evaluate short-term spatial working memory and spontaneous alternation (Conrad et al., 1996). The Y-Maze comprises three identical aisles, each measuring 45 × 12 × 35 cm, arranged at angles of 120 degrees to one another along the medians of an equilateral triangle. The rats were initially placed within one of the three aisles, with their heads oriented toward the intersection point of the three arms. They are then allowed a 5-minute period for free exploration During the test, the recorded parameters include the number of entries into the arms, as well as the number of commonly referred to as series of three successive visits or alternations. (Mandillo et al., 2008).

**Morris Water Maze:** The Morris Water Maze (MWM) was employed to evaluate rats' ability to remember and process spatial information and navigation skills in escaping dirty pond water conditions to reach a hidden platform (7 cm below water surface, 17 cm high). The experimental device was an opaque basin (90 cm diameter, 33 cm height) filled with water (21 cm height) and divided into four quadrants: northeast (NE), southeast (SE), southwest (SW), and northwest (NW). The acquisition phase lasted 4 days with 4 daily trials, locating the platform in the NW quadrant, 2 cm below the water surface. Rats were placed

in the basin, head directed against the wall at one of the four cardinal points (Kahloula et al., 2014). Their time to reach the platform was recorded. A probe test occurred the day after the learning phase, where the platform was removed, and the rats placed in the SE quadrant, with the probe test taking place 2 hours later. A visible platform was positioned in the middle of the NW quadrant, and four 60-second trials from each cardinal point measured the rats' time to reach it.

### Statistical Analysis

In order to determine the differences between the control and experimental groups, the statistical analysis was performed through analysis of variance (ANOVA) using GraphPad Prism 7 software. The comparison of the means and their classification was carried out through Tukey's post hoc test. Significance levels were set at $p<0.05$ to indicate differences as significant, $p<0.01$ to express very significant differences, and $p<0.001$ to represent highly significant differences.

## RESULTS

### Chromatographic analysis results

The chromatographic analysis of the MECL detected the presence of 12 compounds. Predominantly, the composition was characterized by 48.70% of β-turmerone, with detection occurring at 24.67 minutes, and 10.64% of α-turmerone, detected at 25.11 minutes, as portrayed in Figure 2.

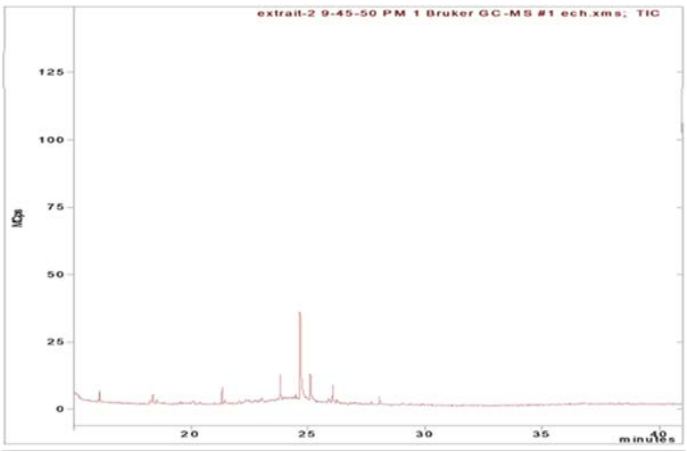


**Figure 2:** Chromatogram of the methanolic extract of the *Curcuma longa* realized by the GC-MS liquid chromatography technique.

### Antioxidant Activity

By using the regression line equation from the linear portion of the graph (Figure 3), we were able to deduce the IC50 value by extrapolating to the 50% inhibition percentage for both MECL and ascorbic acid, as shown in Table 2. The IC50 value deduced from our analysis indicates a notably high level of antioxidant activity exhibited by our extract.

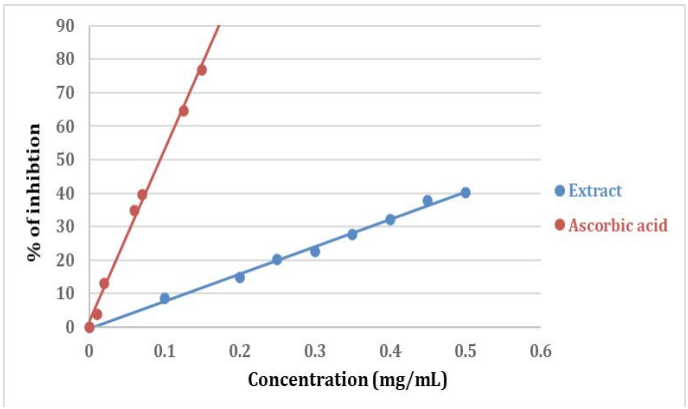


**Figure 3**: Inhibition percentage versus Ascorbic acid and methanolic *Curcuma longa* extract concentration for the DPPH reduction assay

**Table 2:** IC50 for MECL and ascorbic acid

| | MECL | Ascorbic acid |
|---|---|---|
| IC 50 mg/ml | 0.813 ± 0.167 | 0.098± 0.004 |

IC 50. Half maximal inhibitory concentration, MECL. methanolic extract of *Curcuma longa*

### Mortality and Clinical Signs

Regarding the acute toxicity test, force-feeding our extract on male Wistar rats did not induce any mortality within the group force-fed at 2000 mg/kg. On the third day, only one mortality was observed within the group force-fed at 4000 mg/kg, and another one in the group 3000 mg/kg on the seventh day. This leads us to conclude that the No Observable Effect Level (NOEL) dose for this extract stands at 2000 mg/kg. A lethal dose was not identified due to no exhibition of a mortality rate of at least half of the group's total rats. For the control and 2000 mg/kg groups, no variations in clinical signs of toxicity or changes in motor activity were detected. However, in the case of the two groups 3000 and 4000 mg/kg, some disturbances in the rat behavior were observed, including instances of aggression, scratches, and tremors, among others, as depicted in Table 3.

### Biochemical Parameters

The Methanolic Extract of *Curcuma Longa* (MECL) produced no significant alterations in HDL cholesterol levels among the groups. In the case of blood glucose levels, no significant differences were observed between the treated groups and the control groups (Table 4).

### Organ Weights

After 14 days of acute toxicity testing, there were no significant differences observed in the weights of various organs, including the liver, heart, kidneys, and brain, as depicted

**Table 3:** Observation of MECL harmful effects on male Wistar rats

| Days | behavior | posture | scratching | furs aspect | aggressiveness | paralysis | sedation | drowsiness | tremors | asphyxia |
|---|---|---|---|---|---|---|---|---|---|---|
| **Control** | | | | | | | | | | |
| 1 | N | N | N | N | N | N | N | N | N | N |
| 3 | N | N | N | N | N | N | N | N | N | N |
| 5 | N | N | N | N | N | N | N | N | N | N |
| 7 | N | N | N | N | N | N | N | N | N | N |
| 9 | N | N | N | N | N | N | N | N | N | N |
| 14 | N | N | N | N | N | N | N | N | N | N |
| DMSO | | | | | | | | | | |
| 1 | N | N | N | N | N | N | N | N | N | N |
| 3 | N | N | N | N | N | N | N | N | N | N |
| 5 | N | N | N | N | N | N | N | N | N | N |
| 9 | N | N | N | N | N | N | N | N | N | N |
| 14 | N | N | N | N | N | N | N | N | N | N |
| 2000 mg/kg of MECL | | | | | | | | | | |
| 1 | N | N | N | N | N | N | N | N | N | N |
| 3 | N | N | N | N | N | N | N | N | N | N |
| 5 | N | N | N | N | N | N | N | N | N | N |
| 9 | N | N | N | N | N | N | N | N | N | N |
| 14 | N | N | N | N | N | N | N | N | N | N |
| 3000 mg/kg of MECL | | | | | | | | | | |
| 1 | N | N | N | N | N | N | N | N | N | N |
| 3 | N | N | N | N | N | N | N | N | N | N |
| 5 | N | N | N | N | N | N | N | N | P | N |
| 9 | N | N | N | N | N | N | P | N | N | N |
| 14 | N | N | P | N | A | N | N | N | N | N |
| 4000 mg/kg of MECL | | | | | | | | | | |
| 1 | N | N | N | N | N | N | P | N | N | N |
| 3 | N | N | P | N | N | N | N | N | N | N |
| 5 | N | N | N | N | A | N | P | N | P | N |
| 9 | N | AN | N | N | N | N | N | N | P | N |
| 14 | N | N | N | N | N | N | N | N | P | N |

N. normal, AN. abnormal, P. presence, A. aggressive, DMSO. Dimethyl sulfoxide, MECL. methanolic extract of *Curcuma longa*

**Table 4:** Biochemical parameters of male Wistar rats force-fed with MECL and control groups after 14 days of acute toxicity. *

| Biochemical Parameters (g/l) | Control | DMSO | 2000 mg/kg | 3000 mg/kg | 4000 mg/kg |
|---|---|---|---|---|---|
| Glucose | 0.95±0.63 | 0.93±0.39 | 1.03±0.32 | 0.75±0.22 | 0.98±0.20 |
| HDL cholesterol | 1.72±0.62 | 1.71±0.27 | 1.68±0.42 | 1.69±0.30 | 1.75±0.21 |

DMSO. Dimethyl sulfoxide

* Results are represented by mean ± SEM (n=6).

in Figure 4. Furthermore, macroscopic examinations of these organs revealed no significant alterations in color and texture when comparing the control groups to the other groups.

## Anxiety-like Behavior

Assessing anxious behavior in rats can be achieved by measuring the number of central squares visited and the time spent in these central squares. According to Figure 5, the fructose feeding resulted in a significant decrease in the number of central squares visited within the Open Field

test compared to the control group. The Methanolic Extract of *Curcuma Longa* (MECL) exhibited no significant effect when compared to the fructose-fed rats. Co-administering MECL alongside fructose led to a decrease in the number of central squares visited compared to the control group. Regarding the time spent in the center, fructose consumption significantly reduced it ($p<0.01$) compared to the control group. The administration of MECL and losartan considerably increased the time spent in the center; however, this difference is not significant when compared to the fructose group (Figure 5).

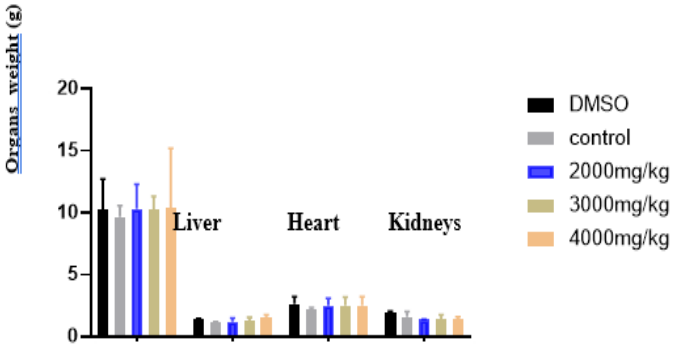


**Figure 4:** Organs weight rats taken after sacrifice after 14 days of acute toxicity of MECL. The results are represented by the mean ± SEM (n = 6). Dimethyl sulfoxide (DMSO).

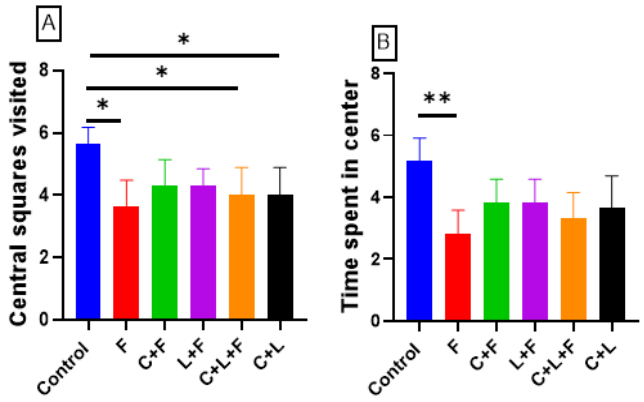


**Figure 5:** Evaluation of the effect of the fructose, the MECL and losartan on the anxiety of rats in open fields. Number of central squares visited (A) and the time spent in the central cells (B) in the open field test. The results are represented by the mean ± SEM (n = 6), the statistical analyses were carried out by unidirectional ANOVA followed by Tukey's post hoc test; the significance level is. * *P* <0.05, ** *P* <0.01, *** *P* <0.001, C. *Curcuma longa*, F. fructose, L. losartan.

The EPM measures the number of entries into the open arms as well as the time spent in them. As illustrated in Figure 6-A, fructose consumption reduced the number of entries ($p<0.05$). Additionally, Figure 6-B shows a significant decrease in the time spent in the open arms compared to the control group. The MECL, losartan and their combination improved the number of entries and the time spent within the open arms. However, these improvements were not significant when compared to the fructose-fed group.

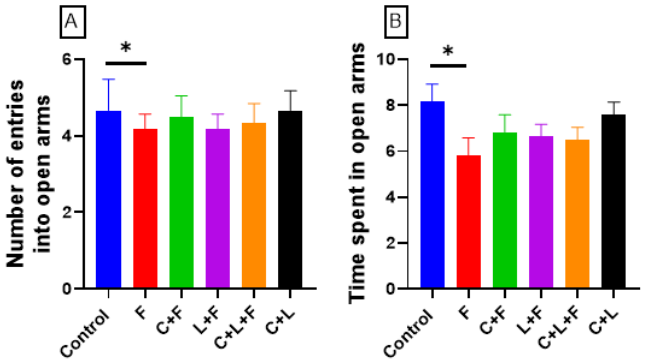


**Figure 6:** Evaluation of the effect of the fructose, the MECL and losartan on anxiety like in the EPM. A: Number of entries into open arms. B: Time spent in open arms. The results are represented by the mean ± SEM (n = 6), the statistical analyzes were carried out by unidirectional ANOVA followed by Tukey's post hoc test; the significance level is. * *P* <0.05, ** *P* <0.01, *** *P* <0.001. C. *Curcuma longa*, F. fructose, L. losartan.

## Depression-like Behavior

The Forced Swimming Test serves as a means to evaluate depressive behavior in rats by measuring the immobilization time (IT). In this context, the fructose consumption resulted in a significant increase in IT ($p<0.01$) compared to the control group. The 10-day treatment with *curcuma longa* and its combination with losartan successfully prevented this increase in the time of immobility observed in the fructose-fed group ($p<0.05$). However, losartan alone had no significant effect in IT compared to the fructose-fed group (Figure 7).

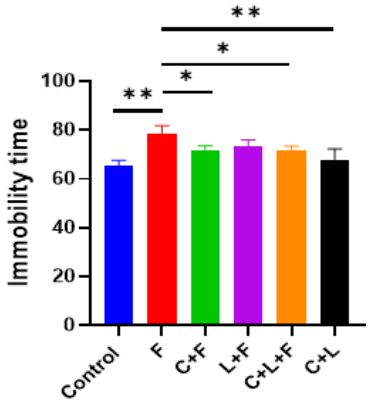


**Figure 7:** Evaluation of the effect of the fructose, the MECL and losartan on the immobility time in the forced swimming test.
The results are represented by the mean ± SEM (n = 6), the statistical analyses were carried out by unidirectional

ANOVA followed by Tukey's post hoc test; the significance level is. * *P* <0.05, ** *P* <0.01, *** P <0.001. C. *Curcuma longa*, F. fructose, L. losartan.

### Memory Activity

To assess this type of memory, The Y Maze test was employed by measuring the percentage of alternation. The fructose consumption had a significant effect (p <0,01) on the alternation percentage when compared to the control group. The treatment with *Curcuma longa* extract, losartan and their co-administration effectively prevented this decrease without signification compared to the control group (Figure 8).

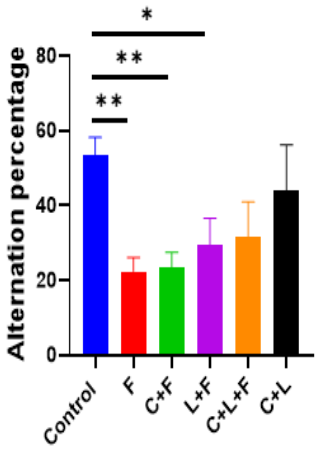


**Figure 8:** Evaluation of the effect of the fructose, the MECL and losartan on the alternation percentage in the Y-MAZE test.
The results are represented by the mean ± SEM (n = 6), the statistical analyses were carried out by unidirectional ANOVA followed by Tukey's post hoc test; the significance level is. * P <0.05, ** P <0.01, *** P <0.001. C. *Curcuma longa*, F. fructose, L. losartan.

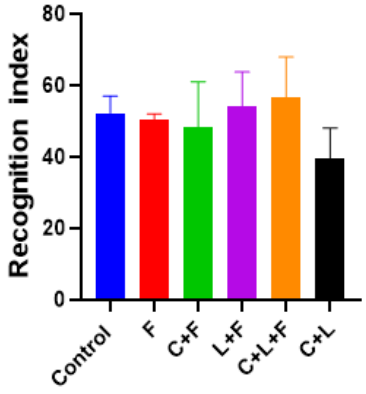


**Figure 9:** Evaluation of the effect of the fructose, the MECL and losartan on the Recognition index in the object recognition test. The results are represented by the mean ± SEM (n = 6). C. *Curcuma longa*, F. fructose, L. losartan.

The long-term recognition memory was evaluated using the object recognition test. The fructose fed group, the *Curcuma longa* and losartan treatments exhibited no significant effect on the recognition index in comparison to the control (Figure 9).

For the Spatial memory ability, The Morris water maze is a test performed to evaluate spatial memory ability. It represents the ability to locate oneself in space, to memorize important places and move optimally. During the probe test, the statistical analyses show that the time spent in the NO (Northwest) quadrant was very significantly lower (p < 0.01) in rats belonging to the Fructose group when compared to those in the Control group. The 10-day treatment with the Methanolic Extract of *Curcuma Longa* (MECL) had no significant impact on this parameter in comparison to the fructose-fed rats, as shown in Figure 10.

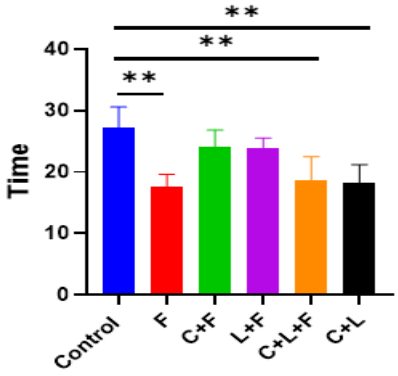


**Figure 10:** Evaluation of the effect of the fructose, the MECL and losartan on the time spent in the quadrant where the platform was located during the acquisition phase. The results are represented by the mean ± SEM (n = 6), the statistical analyses were carried out by unidirectional ANOVA followed by Tukey's post hoc test; the significance level is. ** P <0.05, ** P <0.01, *** P <0.001*. C. *curcuma longa*, F. fructose, L. losartan.

## DISCUSSION

In our study, we first identified that the active ingredient of *Curcuma longa* is sufficiently present in this extract at 60% according to CG-MS, and that the lethal dose of the extract was determined to be higher than 4000 mg/kg. Following one month of fructose feeding, our findings reveal the development of depressive behavior indicated by an increase in immobility time increase and the emergence of anxious behavior, elucidated in the Y-Maze. Furthermore, the harmful effect of fructose on memory is reflected by the inability to memorize different arms of the EPM and a decline in spatial learning performance during the MWM. Notably, our results indicate that a 10-day administration of the MECL as well as the co-administration of MECL

and losartan successfully mitigated the depression-like behavior in high fructose-treated rats.

Regarding the lethal dose of the extract, our study allows us to conclude that it surpasses the doses employed in our study (4000 mg/kg). The NOEL dose for the *Curcuma longa* extract is estimated at 2000 mg/kg. These findings align with previous research, providing consistency with previous studies (Etame-Loe et al., 2019; Gopi et al., 2016; Dadhaniya et al., 2011). The antioxidant activity of the MECL is higher than that of the ascorbic acid, signifying the presence of very high antioxidant potential within our extract. This outcome can be explained by the presence of 60% of terpenes, specifically α-turmerone and β-turmerone in the MECL. These compounds have been recognized for their significant antioxidant properties (Gonzalez-Burgos & Gomez-Serranillos, 2012).

Our results allow us to conclude that there was a development of depressive and anxious behavior in rats subjected to a high fructose diet for the duration of 30 days. The same result was shown in the study of Harrell et al. (2015). Their research revealed that chronic fructose consumption is associated with obesity and is linked to both central and peripheral insulin resistance, which further supports our observations. There is evidence suggesting that central insulin resistance in obesity is closely linked to an increased risk of depression and anxiety (Gancheva et al., 2017). The study carried out by Grillo et al. revealed that a reduction in hypothalamic insulin receptor expression, achieved through the injection of a lentiviral vector into the third ventricle of rats, resulted in an increased immobility time (Grillo et al., 2011). Our investigation delved into the effects of fructose on various aspects of memory through the Y-Maze and Morris Water Maze (MWM) tests. The 30-day fructose-fed group displayed a decrease in spatial learning and memory performance. These findings align with prior research, and the significant associations found are consistent across different durations and doses of fructose consumption. It is evident that fructose intake triggers elevated triglycerides and insulin resistance (Montonen et al., 2007; Ross et al., 2009). Moreover, multiple studies have provided evidence indicating that peripheral insulin resistance is closely associated with memory deficits (Convit, 2005; Ross et al., 2009). Indeed, the direct injection of triglycerides into cerebral ventricles disturbed memory (Farr et al., 2008). Our study demonstrated that a dietary supplementation with the MECL for a duration of 10 days effectively prevented the development of depression induced by fructose consumption. This aligns with the anti-depressant properties of *Curcuma longa* observed in previous research, particularly in studies that administered the compound via bilateral olfactory pathways at doses ranging from 1.25 to 10 mg/kg, as well as in cases of mild chronic unpredictable stress using a dosage of 40 mg/kg (Fan et al., 2019; Xu et al., 2005).

The administration of *Curcuma longa* has been shown to elevate the level of serotonin and dopamine in the hippocampus, which holds notable significance for mood regulation. Interestingly, *Curcuma longa* supplementation has the ability to reduce apoptosis induced by inflammation in mood-regulating regions including the ventromedial prefrontal cortex, by decreasing the presence of apoptotic proteins including Bak, Caspase-3 and Caspase-9. These findings suggest that curcuma's interference with the MAPK-p38 pathway is instrumental in its antidepressant effects (Fan et al., 2019; Alcocer-Gómez et al., 2016). Indeed, *Curcuma longa* can also induce its antidepressant effect by inhibiting enzymes like monoamines oxidase and indolamine dioxygenase (Bhutani et al., 2009). The co-administration of AT1 receptor antagonist, losartan and the MECL prevented depression-like behavior, a result consistent with the findings of Lenart et al. (2019). In their research, they observed a similar antidepressant effect in depressed diabetic rats treated with Losartan at a dosage of 20 mg/kg/day for a period of 2 weeks. Conversely, Costa et al. reported that losartan, administered at a dosage of 30 mg/kg/day for 10 days, did not exhibit an antidepressant effect in rats subjected to chronic stress, as determined through the sucrose preference test (Costa-Ferreira et al., 2019; Morais-Silva et al., 2019; Sahne et al., 2016). This difference in results can be attributed to the use of different tests (FST and sucrose preference test) and the co-administration of MECL in our study which possesses antidepressant properties. Indeed, Losartan is associated with the attenuation of microglia and astrocyte activation, along with an increase in the levels of brain-derived neurotrophic factor (BDNF) and its signaling pathway within the hippocampus (Lenart et al., 2019).

## CONCLUSION

Our findings suggest that the lethal dose of the methanolic extract of *Curcuma longa* is higher than 4000 mg/kg, affirming its safety for use. Moreover, a 10-day dietary supplementation with *Curcuma longa* revealed significant antidepressant effects and improved memory capabilities. This supplementation effectively mitigated depressive behaviors and memory deficits induced by high fructose consumption in rats. Additionally, the combination of the MECL and losartan presents promising potential in preventing memory impairment associated with a high fructose diet. These results can be attributed to the composition of our MECL, containing 60% terpenes, specifically α-turmerone and β-turmerone, which have demonstrated high antioxidative activity. Overall, the collective evidence does emphasize the value of methanolic extract of *Curcuma*

*longa* as a potent therapeutic agent with significant benefits for both mood regulation and memory enhancement.

## ACKNOWLEDGEMENT

We specify that the article is neither published nor submitted to any other journal, It is also recognized that all authors have contributed significantly and that all authors agree with the content of the manuscript is included. we declare that all authors accept the conditions set out in the copyright assignment form is included.

## FUNDING INFORMATION

No financial source.

## DATA AVAILABILITY

The original data from the paper are available from the corresponding author upon reasonable request.

## CONFLICT OF INTERESTS

The contact author has declared that none of the authors has any competing interests.

## NOVELTY STATEMENT

The novelty of this study is the evaluation of the acute toxicity of the methanolic extract of Curcuma longa as well as its preventive antidepressant, antioxidant, and memory activities, in combination or not with losartan, on a hypercaloric diet in female Wistar rats.

## AUTHOR CONTRIBUTIONS

**Sara Brikat** and **Imane Kherrab** contributed equally as principal investigators.
**Yassine Taaifi** contributed in data analyses, final approval of the version. **Laila Ibouzine-Dine**, **Oumaima Abouyala** and **Ilhame Fitah** Contributed in the experimental tasks. **Abdelhalim Mesfioui** and **Aboubaker Elhessni** were responsible for designing the research methodology.

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