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EFFECT OF HYDROALCOHOLIC CINNAMOMUM ZEYLANICUM EXTRACT ON RESERPINE-INDUCED DEPRESSION SYMPTOMS IN MICE

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ABSTRACT

Background: Major depressive disorder is the most common mood disorder that is developed in 17% of the people. By 2020, the disorder will become the second cause of disability in the world. This study was conducted to investigate antidepressant and antioxidant effects of Cinnamomum zeylanicum extract in mice.

Methods: In this study, the male BALB/c mice were used. To induce depression, reserpine was administered and then fluoxetine and C. zeylanicum extract were injected to the mice. To investigate the sedative effect of the drug, rotarod test was used. Forced swim test (FST) was used to investigate depression. The free radical DPPH method was used to determine antioxidant activity of extract and the Folin–Ciocalteu method to determine phenolic, flavonoid, and flavonol compounds. Data analysis was conducted by SPSS 21.

Results: In the FST, there was a significant difference in immobility duration between 7 groups. C. zeylanicum extract in all doses decreased immobility duration in the FST. There was no significant difference in immobility duration between C. zeylanicum-treated and fluoxetine-administered groups. The antioxidant activity (IC50) of the extract was 12.82 and for phenolic, flavonoid, and flavonol compounds, it was 264.37, 27.14, and 26.25 mg/g of dried extract respectively.

Conclusion: C. zeylanicum extract, improved depression in the mice, but its effect was not dose dependent. The antidepressant effect of C. zeylanicum was also equal to that of fluoxetine. This extract has potent antioxidant activity and the antidepressant effect of this extract is likely due to its antioxidant property.

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Introduction

Major depressive disorder (MDD) is considered an important disorder of the current community. The lifetime prevalence of depression is high [approximately 17% (5-17%)] and is considered to be the most common mood disorder whose incidence rate is two times higher in women than men [1,2]. This disorder is currently the fourth cause of disability but will become the second factor by 2020 [3,4]. MDD refers to a collection of symptoms that impairs the function of patients and recurs in most cases [5]. The symptoms of MDD include depressed mood and loss of interest decreases or increases sleep and appetite, difficulty in concentrating, feeling guilty and worthless, constantly thinking about death, suicidal idea, and psychomotor retardation or agitation. The first episode is 40% likely to occur within the following two years [6]. Following two episodes, the risk of recurrence in the next five years is about 75% [7].

Latest evidence indicates that the incidence rate of major depression is increasing in the people aged under 20 years old [8]. Treatments can be psychotherapeutic, pharmacotherapeutic, or a combination of them [9].

The characteristics of an ideal antidepressant drug are: fast onset, moderate half-life, minimal side effects, minimum toxicity at high doses, and fewer interactions [10].

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Different classes of drugs are used for treating depression [11] out of which SSRIs (Selective Serotonin Reuptake Inhibitors) are first-line treatments because of fewer side effects [12].
Although numerous drugs are used for treating depression, most of them cause certain adverse drug side effect [13, 14].
Fluoxetine is an old and the first SSRI that has been marketed. This drug leads to decreased symptoms of depression after being taken for 4-6 weeks [15].
Because chemical drugs are usually associated with numerous adverse side effects and psychotherapy is relatively time-consuming and costly, using plants for treatment or as complementary therapy can be a highly valuable achievement [16]. We know that no single treatment is effective for the depressed patients [17], thus evaluation of alternative therapies seems to be necessary. Plant-based extracts are one of the most attractive new pharmaceutical sources and have been demonstrated to lead to promising outcomes in treating depression [18, 19].
Cinnamon is one of the famous and oldest known medicinal plants such that its history dates back to 2700 years B.C [20]. Cinnamon is the dried bark of Cinnamomum zeylanicum Blumeo stem from family Lauraceae or laurel with latin name of C. zeylanicum and English name of common true cinnamon or Cinnamomum tree ceylan cinnon [21]. C. zeylanicum is an evergreen tree 8-12 m high, with a dense and wide covering crown, short and green-face stem with a bulky rooting system that has many endings [22]. Bark is the pharmaceutical part of C. zeylanicum [21].
C. zeylanicum bark contains 1% of C. zeylanicum essential oil that is the only important part of this plant and is obtained by water distillation [23].
This plant has been used to treat different diseases such as diarrhea, anger and aggression, joint inflammation, common cold, fever, and diabetes as well as to decrease insulin resistance [20].
According to Avicenna's Cannon and Abureyhan’s Sidneh, C. zeylanicum is used in many conditions such as indigestion, diarrhea, dyspnea, speed up circulation, relieving postpartum pain, facilitating menstruation, treating insomnia, inducing relaxation, and increasing visual acuity [24]. In addition, C. zeylanicum is exhilarating and anti-anxiety and anti-obsessive, and has long been used to treat insanity [25]. Its physiological activity is due to its essential oil and tannin. Cinnamaldehyde is the main compound of C. zeylanicum essential oil, and the essential oil derived from its bark contains 55-57% cinnamaldehyde and 15-18% eugenol [26].
The bark of this plant contains 0.5-2.5% essential oil whose main compounds are cinnamaldehyde, eugenol, and trans-cinnamic acid. Besides that, its essential oil contains other propane phenyl compounds such as hydroxycinnamaldehyde, ortho-methoxycinnamaldehyde, cinnamyl alcohol, and its acetate as well as terpenes. [27] Cinnamaldehyde is one of the compounds of C. zeylanicum essential oil that stimulates the central nervous system (CNS) in low doses, exerts palliative effect in high doses [28], and has sedative effect on both animals and humans [23].
It has recently been demonstrated that C. zeylanicum is used for relaxation and prevention of anger and increasing appetite in addition to gastrointestinal diseases [23,28]. Despite such effects, pharmacological and toxicological studies have not yet demonstrated any specific risk due to consumption of cinnamon in human [26], but its use is forbidden in pregnancy [21].
No study has yet been conducted to investigate the effect of C. zeylanicum in treating depression, but the effects of different medicinal plants such as Lavandula offficinalis, lavender, mint, chamomile, rose oil, geronayon oybean, garlic, sweetheart, red panax ginseng, and Alafia multiflora Stapt in treating major depression have been investigated [23, 29-37].
Several researches have investigated the effects of C. zeylanicum in several conditions such as: treating fungal infections and diabetes, insulin resistance, Bacillus cereus growth, and dysmenorrhea, in controlling chronic pain as well as on hypothalamic-pituitary gonadal axis. The antioxidant effect of this plant has also been studied [20, 24, 26, 27,38-44]. With regards to the above-mentioned and because the antidepressant effect of C. zeylanicum has not yet been studied, we investigated this effect in mice to contribute to treatments for major depression.

Materials and Methods

This interventional-experimental study was conducted on BALB/c in 2015 mice at Medicinal Plants Research Center of the Shahrekord University of Medical Sciences after the Ethics Committee of this university provided necessary approval (Approval number: 92-4-14). C. zeylanicum was also extracted at this center. A total of 56 mice weighing 25-30g were purchased from Pasteur Institute of Iran and assigned to seven groups of eight each according to the formula and the advice of a statistician at 95% confidence interval. The mice were housed at (23±2) °C temperature and 12-h lightness/12-h dark cycle and fed with the animal food (Pasteur Institute of Iran). The mice had free access to food and water except for when they were being tested. Each animal was tested only once [20].
To avoid confounders, all mice were BALB/c, were purchased from the Pasteur Institute of Iran, and weighed 25-30g. The inclusion criteria for mice were being BALB/c, weighing 25-30 g, and being alive. The only exclusion criterion for the mice was death during the study.
C. zeylanicum that is abundantly cultivated was first prepared and then approved in the Herbarium of the Medicinal Plants Research Center of the Shahrekord University of Medical Sciences. To prepare the hydroalcoholic extract, dried C. zeylanicum was first pulverized and then passed through 40 and 80-mesh sieve. One hundred gram of the pulverized C. zeylanicum was poured into 200ml of methanol 80% and extracted by percolation for 72 hours. After filtering the extract using Whatman filter

paper, we dried it using a vacuum distillator (Memmert) available in the Medical Plants Research Center of the Shahrekord University of Medical Sciences at 35-40°C so that the extract was concentrated [45].

Different doses (50, 100, 200, and 400 mg/kg) of the extract were prepared by injection of normal saline. For this purpose, with regards to the mice's weights (approximately 30g), to prepare 50, 100, 200, and 400 mg/kg doses of the extract, 75, 150, 300, and 600mg of the dried extract, respectively, was separately weighed using a very sensitive digital scale (Sartorius) to the nearest 0.1g and poured into 15-ml falcon tube. Then, the content of each tube was increased to 10ml by injection of normal saline and the dried extract was thoroughly dissolved in the injected sterile normal saline using a lantern shaker (LS 100) such that no deposition remained in the bottom of the falcon tubes. According to calculations, the injection of 0.2 ml of the solution was adequate to prepare the extract in the dose of interest. The purpose of selecting these doses was to study the extract in a wide range of concentrations.

First, the mice were divided into 7 groups of 8 each.

Five mg/kg of reserpine was intraperitoneally injected for inducing depression 18 hours before tests [31]. Then, the treatment for depression was conducted by intraperitoneal injection of 0.5mg/kg body weight (BW) of fluoxetine and C. zeylanicum extract in 50, 100, 200, and 400mg/kg BW in different groups. To conduct this study, rotarod test and forced swim test (FST) were also performed. The FST is good screening tool for depression in mice with reliability and predictive value [46-48].

According to Seligman's Theory of Learned Helplessness, if animal is exposed to constant stress and cannot get rid of it, it will gradually lose hope of escaping from this situation and stop its mobility and activities and become helpless and motionless [49].

For this purpose, the mice were separately and gently placed in an open cylinder with 12-cm diameter and 25-cm height that was filled with water to a height of 19 cm so that the mice were forced to swim. In this condition, the mice were forced to paddle to prevent drowning. The first two minutes were specified to the mice's adjustment to the environment, and then, within the following 4 minutes, the durations of paddling and immobility of the mice were measured and recorded. Conventionally, the stopping of the mouse’s hands and feet movements is considered immobility [31, 50]. To measure immobility duration, the sum of the durations when the mouse was motionless was recorded within a specific period. Increased duration of immobility was considered to represent depression and its decrease was considered the efficacy of the treatment for depression [31]. FST was conducted 60 minutes after intraperitoneal administration of the C. zeylanicum extract [30].

Because the assessment of depression in FST is dependent on the motor activities of the mice, in the current study, rotarod test was conducted to investigate sedation and motor side effects of the used drugs before and after administrations. In this test, the mice’s motor activities were tested by rotarod instrument (Harvard, England) [51]. Because sedation has adverse effect on motor activities [52] and the assessment of depression in FST was dependent on the motor activities of the mice, we investigated the presence of this problem in our samples.

This device consists of a rotational dipped rod of 6 cm in diameter, divided into four equal parts by 5 discs so that four mice are simultaneously placed on the instrument. The rotary rod was located 25 cm away from the magnetic base with a magnetic key to stop the time. The rotary rod began to rotate at a speed of 5 rpm, and during spin, its rotational speed reached a maximum of 10 rpm. The motor coordination of the mice was measured based on the duration of their balance on the rotational rod, and as soon as the mouse fell to the magnetic base, the magnetic key stopped the time, and the time during which the mouse maintained its balance was recorded. This test was conducted on all mice before and after injection of the drugs.

One day before conducting the tests, the mice were adjusted to the instrument to learn how to maintain balance on the rotational rod. The mice that were able to maintain their balance for 60-100 seconds on the rod were selected to undergo rotarod test and FST after administrations. On the day of the test, if the mouse maintained its balance on the rod for five minutes (300s) the experiment was stopped and the mouse was considered to have normal motor activities [51,52].

Before administrations, rotarod test was conducted on the mice in all groups per the above-mentioned procedure and all mice were able to maintain balance on the rod for five minutes. This test was conducted again on all mice 30 minutes after administrations. In addition, comparison of duration of balance maintenance on the rod among control, negative control, positive control, and all C. zeylanicum extract-treated groups demonstrated that whether the conducted administrations were effective or ineffective on the mice's motor activities, and therefore the sedative effects of the administered drugs could be investigated [51,52].

After administrations and rotarod test, FSM was separately conducted on all mice. These two tests are considered standard tests across the world whose validity and reliability have been confirmed in several studies conducted in Iran and other countries. All above-mentioned tests and experiments have been accepted to be standard tests worldwide and used in studies conducted in Iran and other countries to measure these variables.

In addition, the antioxidant activity and phenolic, flavonoid, and flavonol content of C. zeylanicum extract were measured to determine the biochemical causes of (lack of) effect of this extract on depression symptoms in mice. For this purpose, the antioxidant activity of dried C. zeylanicum extract was measured by the protocol of Ghasemi et al. to determine free radical-scavenging activity using the DPPH:

1. First, 1mg/ml of the extract stock, 1mg/ml of BHT, and 0.1mM of the DPPH (all dissolved in ethanol) were prepared;
2. Using the prepared stocks, we prepared 2ml of the extract and 2ml of BHT in 5-100µg (10, 20, 40, 60, 80, and 100µg) concentrations;

3. Two ml of the DPPH was added to all concentrations of the extract or BHT and the resulting solution left in the dark for 15 min. In addition, control tube containing 2ml of ethanol and 2ml of the DPPH was prepared alongside other samples;
4. Fifteen minutes later, the spectrophotometer at 517-nm wavelength was set at zero using ethanol blank and then the absorbance of the samples was read. IC50 was calculated by the formula below:
5. By plotting the curve, the concentration in which 50% of the DPPH radicals were neutralized was derived; X axis represented the extract concentration and Y axis did the inhibition rate; and
6. The concentration in which 50% of the DPPH radicals were neutralized was considered IC50 and expressed as mg of the dried extract, mM of the antioxidant substance, or the number of the neutralized DPPH molecules per a molecule of antioxidant substance [53].

Investigating the antioxidant compounds of C. zeylanicum dried extract using Folin–Ciocalteu method:
In this method, phenolic, flavonoid, and flavonol content of the extract were measured according to the protocols below:

1. Phenol blank was prepared with addition of 0.5ml of Folin-Ciocalteu reagent to 0.5 ml of sodium carbonate
2. Flavonoid blank and flavonol blank were prepared with addition of 0.5ml of the extract solution to 3.5 ml of distilled water;
3. Phenolic compounds [gallic acid equivalent (GAE)/g of extract] were determined by colorimetric method using Folin-Ciocalteu reagent. Standard gallic acid in different concentrations was dissolved in 60% methanol. Then, 0.1ml of each sample was transferred to a tube containing 0.5ml of 10% Folin-Ciocalteu as reagent. The solutions were left at room temperature for 10 minutes and then 0.4ml of 7.5% carbonate was added to them. The resulting solutions were left at room temperature for 30 minutes and then their absorbance was read using a spectrophotometer (Unico UV 2010) at 765nm wavelength in triplicate.
   To measure total phenolic content, 0.01-0.02µg of extract was added to 60% methanol to a final volume of 10ml. Then, total phenolic content (mg GAE/g of extract) was determined using Folin-Ciocalteu reagent. 0.1ml of the extract was used as the standard solution [54].
4. Flavonoid content (mg rutin equivalent/g of extract) was determined by aluminium chloride colorimetric method using Folin-Ciocalteu reagent.

For this purpose, different (25, 50, 100, 250, and 500ppm) concentrations of standard rutin in 60% mthanol were prepared and then, 1 ml of each sample was transferred to a tube containing 1 ml of 2% aluminium chloride. Six ml of 5% potassium acetate was added to the solution of each tube and 40 minutes later, optical density read at 415nm wavelength. The levels of the standard solution in different concentrations were measured in triplicate. To measure total flavonoid content, 0.01-0.02µg of the extract was added to 60% methanol to a final volume of 10ml. Then, total flavonoid content (mg rutin equivalent/g of extract) was determined by aluminium chloride colorimetric method. 0.1ml of the extract was used as the standard solution. Optical density was read at 440nm wavelength 2.5 hours later [54].

5. Flavonoid content (mg rutin equivalent/g of extract) was determined by sodium acetate colorimetric method using Folin-Ciocalteu reagent.

First, different (25, 50, 100, 250, and 500parts/million) concentrations of standard rutin in 60% mthanol were prepared and then, 1 ml of each sample was transferred to a tube containing 1 ml of 2% aluminium chloride. Six ml of 5% sodium acetate was added to the solution in each tube and 40 minutes later, optical density read at 415nm wavelength. The levels of the standard solution in different concentrations were measured in triplicate. To measure total flavonol content, 0.01-0.02 µg of the extract was added to 60% methanol such that the volume reached 10 ml. Then, total flavonoid content (mg rutin equivalent/g of extract) was determined. 0.1 ml of extract was used as the standard solution. Optical density was read at 440nm wavelength 2.5 hours later [54].
The mice were assigned to seven groups of eight each as follows:

1. Control group: The mice that were not administered with reserpine, C. zeylanicum extract, or fluoxetine and underwent FST only;
2. Negative control: The mice that were administered with 5mg/kg BW of reserpine to induce depression and then did not receive C. zeylanicum extract or fluoxetine but underwent FST;
3. Positive control: The mice that were administered with 5mg/kg BW (Body Weight) of reserpine to induce depression and then were administered with 0.5mg/kg BW of fluoxetine, a standard antidepressant agent, and underwent FST; and

4-7. C. zeylanicum extract-treated groups: The mice that were administered with 5mg/kg BW of reserpine to induce depression and then were administered with 50, 100, 200, and 400 mg/kg BW of C. zeylanicum extract dissolved in injected sterile normal saline, as the studied treatment, and underwent FST.

All administrations were intraperitoneal and only control group were not administered with reserpine and only underwent FST. Data analysis was conducted by ANOVA and Kruskal-Wallis test in SPSS 21.

Results

This study was conducted to investigate the effect of C. zeylanicum extract on reserpine-induced depression symptoms in mice. Rotarod test was conducted in all groups before and after administrations. In this test, before administrations, all mice could maintain their balance on the rotational rod for at least 300s (cut-off time). Therefore, there was no significant difference in rotarod test among different groups before administrations and all mice were considered to have normal motor activities, and the test was stopped (Table 1).

The results of rotarod test after administrations were as follows (Table 2):

The results of ANOVA ($p=0.634$) and Kruskal-Wallis test ($p=0.614$) demonstrated that there was not any significant difference in duration of balance on the rotational rod among different groups, suggesting that reserpine did not have any sedative effect on the mice.

The results on the duration of the mice's paddling were as follows (Table 3):

The results of ANOVA ($p<0.001$) demonstrated that there was a significant difference in duration of the mice's paddling among different groups, which will be explained with regards to the multiple/post hoc group comparisons in ANOVA.

The findings on the duration of the mice's immobility (the criterion of efficacy or inefficacy of treatment with antidepressant in different groups) were as follows:

The results of ANOVA and Kruskal-Wallis test ($p<0.001$) demonstrated that there was a significant difference in duration of the mice's immobility in FST among different groups.

As already mentioned, in rotarod test, according to ANOVA ($p=0.634$) and Kruskal-Wallis test ($p=0.614$), there were no significant difference in the duration of balance maintenance on the rotational rod among different groups.

To explain significant difference in FST among different groups, multiple/post hoc group comparisons in ANOVA were used. The results are as follows:

The results on duration of paddling in FST are as follows:

Paddling duration in control group was significantly different compared to negative control and positive control groups ($p<0.001$). This duration was not significantly different compared to the groups treated with 50 and 100mg/kg BW of C. zeylanicum extract ($p=0.763$ and 0.305, respectively).

But, paddling duration in control group was significantly different compared to the groups treated with 200 and 400mg/kg BW of C. zeylanicum extract ($p=0.024$ and 0.011, respectively).

Duration of paddling in FST in negative control group was significantly different compared to all other groups ($p<0.001$). In addition, duration of paddling in FST in positive control group was significantly different compared to control and negative control groups ($p<0.001$) but was not significantly different compared to the groups treated with 50, 200, 100, and 400mg/kg BW of C. zeylanicum extract ($p=0.071$, 0.326, 0.940, and 0.988, respectively).

In addition, the duration of paddling in FST in the group treated with 50mg/kg BW of C. zeylanicum extract was not significantly different compared to control, positive control, and the groups treated with this extract in other doses but was significantly different compared to the negative control group ($p<0.001$). This duration was significantly different between the group treated with 100mg/kg BW of C. zeylanicum extract and negative control group ($p<0.001$) but not the other groups.

The duration of paddling in the group treated with 200mg/kg BW of C. zeylanicum extract was significantly different compared to control and negative control groups but not to other groups. This duration was significantly different between the group treated with 400mg/kg BW of C. zeylanicum extract and control and negative control groups ($p<0.001$) but not the other groups.

The results on immobility duration in FST are as follows:

Immobility duration in control group was significantly different compared to negative control and positive control groups and the groups treated with 200 and 400mg/kg BW of C. zeylanicum extract ($p<0.001$) but not the groups treated with 50 and 100mg/kg BW of C. zeylanicum extract.

The duration of paddling in negative control group was significantly different compared to all other groups ($p<0.001$). This duration in positive control group was significantly different only compared to those in control and negative control groups ($p=0.004$). This duration in the groups treated with 50 and 100mg/kg BW of C. zeylanicum extract was significantly different only compared to that in negative control group ($p<0.001$).

The durations of paddling in the groups treated with 200 and 400mg/kg BW of C. zeylanicum extract were significantly different compared to control and negative control groups ($p<0.035$ and 0.017, respectively).

The antioxidant activity of C. zeylanicum dried extract was measured by the protocol of Ghasemi et al. to determine free radical-scavenging activity using the DPPH. In this method, after the above-mentioned procedures, IC50 was calculated by the Equation 1-4:

Through plotting the curve, the concentration in which 50% of the DPPH radicals were neutralized was derived; X axis represented extract concentration and Y axis did the inhibition rate;

The concentration in which 50% of the DPPH radicals were neutralized was considered IC50 and expressed as mg of the dried extract, mM of the antioxidant substance, or the number of the neutralized DPPH molecules per a molecule of antioxidant substance.

All above values of optical density were derived using spectrophotometer at 570nm wavelength. According to the Equation 4-1 and the plotted curve, IC50 was 12.82µg/µl.

IC50=12/82(µg/µl)

Therefore, dried C. zeylanicum extract in 12.82µg/µl can inhibit the activity of 50% of the DPPH molecules (an oxidant compound). Taking this into account and because this extract in 12.82µg/µl inhibits the oxidant activity of approximately 90% of the DPPH molecules, then this extract has a potent antioxidant activity which can be the cause of its antidepressant effect (Table 4 and Figure 1).

The antioxidant compounds of dried C. zeylanicum extract

As already mentioned, the antioxidant compounds of C. zeylanicum extract are as follows:

1. Phenolic compounds (mg GAE/g of extract) were colorimetrically measured using Folin-Ciocalteu reag.
2. Flavonoids (mg rutin equivalent/g of extract) were determined by aluminium chloride colorimetric method using Folin-Ciocalteu reagent.
3. Flavonols (mg rutin equivalent/g of extract) were determined by aluminium chloride colorimetric method using Folin-Ciocalteu reagent.

Accordingly, and as the tests indicated, the amounts of antioxidant compounds in this extract are as follows:

- Phenol: 264.37 (mg/gr)
- Flavonoid: 27.14 (mg/gr)
- Flavonol: 26.25 (mg/gr)

Discussion

Today, depression is a common and severe mental disorder in communities and one of the most common chronic diseases. Patients with depression may become severely hopeless such that up to 75% of them feel suicidal and around 25% of them attempt suicide [1].

Currently, the antidepressants from SSRIs family especially fluoxetine are among the most commonly used drugs for depression. Because antidepressants begin to exert effect around 4-6 weeks after onset of treatment, medicinal plants can be considered effective treatments to improve pharmacologic outcomes in the early weeks of treatment with SSRIs because they are more welcomed by patients and lead to comparatively fewer side effects. Therefore, tendency to use herbal drugs has recently increased.

C. zeylanicum is one of the medicinal plants that has long been used in traditional medicine. The physiological effect of this plant is due to the essential oil and tannin. The main compound of C. zeylanicum is cinnamaldehyde and the essential oil of its bark contains 55-57% cinnamaldehyde and 15-18% eugenol. Pharmacological and toxicological studies have not yet reported any specific risk due to taking C. zeylanicum in human [40]. Cinnamaldehyde in low doses stimulates the CNS and in high doses exerts palliative effects [27].

C. zeylanicum is used to improve joint pains, toothache, common cold, influenza, diarrhea, cystitis, and respiratory problems, to manage fever, to treat menstrual problems, to prevent severe anger and aggression as well as to treat vaginal infections caused by Candida albicans, stomach ulcers caused by Helicobacter pylori, and food poisoning caused by Escherichia coli [20]. This is also used to treat many disorders such as indigestion, halitosis, and dyspnea, to speed up circulation, to relieve postpartum pain, to facilitate menstruation, to treat insomnia, to induced relaxation, to increase visual acuity, to stimulate spermatogenesis, to decrease blood sugar as well as to increase high-density lipoprotein and decrease low-density lipoprotein in the blood [23, 24]. In addition, C. zeylanicum is exhilarating, anti-anxiety, anti-obsessive, and has long been used to treat insanity [25].

Because the sedative, palliative, anti-anxiety, and anti-obsessive effects of C. zeylanicum have already been studied, we were encouraged to study the antidepressant effect of this plant using FST and its sedative effect using rotarod test.

Very few studies have been conducted on the effect of C. zeylanicum antidepressant effect and our study is the first study to address this effect of C. zeylanicum extract after induced depression.

C. zeylanicum extract in all doses significantly improved depression symptoms compared to negative control group where depression was induced by parenteral reserpine alone and no antidepressant was administered, because in FST, injection of this extract significantly decreased immobility duration compared to negative control group. However, the antidepressant effect did not increase with increasing C. zeylanicum extract dose because no significant difference was seen in immobility duration among the groups treated with 50, 100, 200, and 400 mg/kg BW of this extract, and therefore its antidepressant effect was not dose dependent.

The effect of C. zeylanicum in treating depression symptoms in the mice was not dose-dependent because no significant difference in immobility duration in FST was seen among the groups treated with the extract in different doses.

Besides that, the effects of C. zeylanicum and fluoxetine on depression in the mice were not significantly different because in FST, there was no significant difference in immobility duration between C. zeylanicum-treated and fluoxetine groups. It can therefore be argued that the effect of C. zeylanicum in treating depression in the mice was equal to that of fluoxetine.

The effects of C. zeylanicum extract in 50 and 100mg/kg BW doses on the mice's immobility duration in FST were not significantly different compared to control group where depression was not induced and no treatment for depression was given.

But C. zeylanicum extract in 200 and 400mg/kg BW significantly decreased immobility duration in FST compared to control group.

In addition, fluoxetine was significantly effective in treating the induced depression symptoms in the mice because this drug significantly decreased immobility duration in the FST. There was no significant difference in improvement of depression symptoms between C. zeylanicum extract and fluoxetine because there was no significant difference in immobility duration in FST between the group treated with fluoxetine and those treated with C. zeylanicum extract in different doses.

It is worth mentioning that none of the used drugs consisting of reserpine, fluoxetine, and C. zeylanicum extract (in all doses) exerted significant sedative effect on the mice because in rotarod test, there was no significant difference in the duration of balance maintenance on the rotational rod among different groups.

Besides that, the IC50 of C. zeylanicum extract in the tests to determine its antioxidant activity was derived 12.82µg/µl; more clearly, this extract in 12.82µg/µl concentration can inhibit the antioxidant activity of 50% of the DPPH molecules and therefore has a potent antioxidant activity. The antioxidant compounds of C. zeylanicum extract, in Folin-Ciocalteu method, were phenol (264.37mg/g), flavonoid, (27.14mg/g), and flavonol (26.25 mg/g). Such high amounts of antioxidant compounds cause this extract to exert potent antioxidant activity, which can be the cause of its antidepressant effect.

There are many assumptions about the causes of depression, for example monoamine theory and oxidative stress hypothesis. One study has suggested the potential serotonergic effect of C. zeylanicum [55]. In another, the antidepressant and antianxiety effects of C. zeylanicum essential oil have been mentioned and it has been suggested that these effects are due to high level of antioxidant activity of essential oil [56]. In our study, we evaluated the antioxidant activity of hydroalcoholic extract of C. zeylanicum.

Conclusion

Taken together, the present study demonstrated that C. zeylanicum was significantly effective in treating induced depression symptoms in mouse that potentially may be due to containing large amounts of antioxidant compounds, i.e. phenols, flavonoids, and flavonols, in its extract and therefore exerting potent antioxidant effect. Antidepressant effect of this extract was not dose dependent

Limitations of the study

Because of the short longevity and sensitivity of mice, many of them died in transferring and the Research and Technology Deputy of the university provided necessary funding to purchase a number of mice from the Pasteur Institute of Iran again. Obviously, following administration, a number of the mice died and therefore two additional mice (a total of 10 mice) were assigned to each group. Fortunately, at most two mice in each group died and therefore rotarod test and FST were conducted on eight mice in each group.

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Figure and Tables:

Table 1. Results of rotarod test after administrations in different groups

p-value	Standard deviation	Mean duration time in rotarod test (second)	Number	Groups
0.634	13.78	279.25	8	1 (control)
	26.38	273	8	2 (negative control)
	21.20	274.25	8	3 (positive control)
	11.7	290.75	8	4 (50mg/kg extract)
	15.92	284.75	8	5 (100 mg/kg extract)
	28.75	278.62	8	6 (200 mg/kg extract)
	20.62	283	8	7 (400 mg/kg extract)
	20.29	280.52	56	Total

Table 2. Results of paddling duration in forced swim test

p-value	Standard deviation	Mean paddling time (second)	Number	Groups
	9.34	95.75	8	1 (control)
	18.1	63.25	8	2 (negative control)
	16.94	122	8	3 (positive control)
	5.04	104.5	8	4 (50mg/kg extract)
	7.21	109	8	5 (100 mg/kg extract)
	7.53	115.75	8	6 (200 mg/kg extract)
	12.71	117.5	8	7 (400 mg/kg extract)
<0.001	21.82	103.96	56	Total

Significant p-value: $p < 0.05$

Table 3. Results immobility duration in forced swim test

p-value	Standard deviation	Mean immobility duration time (second)	Number	Groups
	9.34	144.25	8	1 (control)
	18.1	176.75	8	2 (negative control)
	18.19	119.25	8	3 (positive control)
	5.04	135.5	8	4 (50mg/kg extract)
	9.81	128.5	8	5 (100 mg/kg extract)
	7.53	124.25	8	6 (200 mg/kg extract)
	12.71	122.5	8	7 (400 mg/kg extract)
<0.001	22.03	135.86	56	Total

Significant p-value: $p \leq 0.05$

Table 4. The concentration of DPPH and percentage of its inhibition due to addition of Cinamommum zeylanicum extract in different concentrations

Inhibited DPPH (%)	DPPH concentration (µgr/µl)	Extract concentration (µgr/µl)
4.8	549	0.5
6.75	538	1
12.8	503	2.5
22.8	445	5
40.9	341	10
49.2	293	12.5
60.13	230	15
66.03	196	17.5
78.03	125	20
88.9	64	25

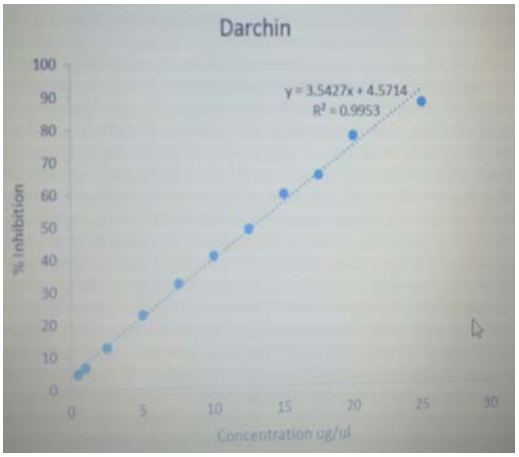

Figure 1. Inhibition of DPPH oxidant activity by Cinamommum zeylanicum in different concentrations