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Investigation of anxiolytic effects of ethanol extract from banana peel (*Musa paradisiaca* L. var Semeru)

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ABSTRACT: *Musa paradisiaca* L. var Semeru (MPS) is a banana with thick skin that produces unused waste. The potential anxiolytic effect of banana peel ethanol extract (BPE) is explored in this study. There were seven groups in this study, namely the normal control group, CMC Na, alprazolam (0.4 mg/kg), tryptophan (270 mg/kg), 5-HTP (18 mg/kg), BPE (140, 280 mg/kg). The number of rats per group in the light-dark box (LDA) test was 4. Meanwhile, the number of rats per group in the elevated plus maze (EPM) test was 6. BPE (140 and 280 mg/kg) was given to rats one hour before the anxiolytic test using a LDA and EPM. BPE (140 and 280 mg/kg) did not significantly increase entries and time spent in the light chamber of the light-dark box. In addition, it also did not significantly affect entries and time spent in open arms in the elevated plus maze. Referring to the LDA and EPM tests, the ethanol extract of MPS did not significantly reduce anxiety.

KEYWORDS: Anxiety; banana extract; elevated plus maze; light-dark activity.

1. INTRODUCTION

In times of peril, anxiety is a common emotion regarded as a component of the response to survival. However, there are numerous situations where having anxiety is maladaptive or excessive and indicates a psychiatric disease in contrast to major depression, which is characterized by core symptoms of sad mood or loss of interest. The concept of excessive fear and concern as core symptoms of anxiety as a psychiatric condition is growing [1].

The emotions perceived as anxiety are produced mainly by the limbic system of the brain. The cingulate and parahippocampal gyri, hippocampus, amygdala, septum, and hypothalamus are specific limbic system organs. It is believed that various affective illnesses, such as anxiety disorders and major depression, are caused by the overactivity of limbic areas and the loss of inhibitory control by the executive portions of the brain [2].

Neurons that originate in the raphe nuclei of the brain stem and diffusely propagate throughout the brain employ 5-HT predominantly as an inhibitory neurotransmitter. Abnormalities may influence anxiety disorders in the release and uptake of serotonin at presynaptic autoreceptors (5-HT1A/1D), the serotonin-reuptake transporter (SERT) site, or the action of 5-HT at postsynaptic receptors (e.g., 5-HT1A, 5-HT2A, and 5-HT2C). Greater 5-HT activity is thought to decrease norepinephrine activity in the locus ceruleus, suppress the defense/escape response via the periaqueductal gray area, and decrease the release of corticotropin-releasing factor from the hypothalamus. The selective serotonin reuptake inhibitors (SSRIs), which are effective in preventing the symptoms of panic and anxiety, acutely raise 5-HT levels by inhibiting the SERT to increase the amount of 5-HT available postsynaptically [3].

A precursor to serotonin production, tryptophan is a necessary amino acid. In this case, tryptophan must be consumed through diet because the body cannot produce it. As a result, dietary factors that affect blood levels of tryptophan and other amino acids might vary tryptophan uptake in the brain, resulting in the rate of serotonin production. Previous studies on 25 healthy young adults for four days showed that high

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tryptophan consumption (>10 mg/kg body weight/day) can significantly reduce anxiety scores when compared to low tryptophan consumption (<5 mg/kg body weight/day) [4]. A systematic review shows that giving tryptophan supplements of 0.14-3 grams/day can improve mood [5].

Bananas are known to improve mood because of their serotonin content, but the serotonin in bananas cannot cross the blood-brain barrier [6]. Moreover, bananas contain tryptophan as the primary source of peripheral and central serotonin [7]. Giving food fortified with 20% banana peels to 30 students can improve their mood after 30 minutes of administration [8]. Moreover, administering 70% acetone extract from the banana peel (*Musa sapientum* L.) on rats can provide an antianxiety effect [9]. *Musa paradisiaca* L. var. Semeru (MPS), an Indonesian variety of bananas with thick skin, has the potential to be investigated as an antianxiety treatment. This excellent Lumajang Regency banana type is typically used to make banana chips. MPS peel is currently considered a waste with no market value. The local community's living level is anticipated to rise with MPS peel as a nutraceutical with antianxiety properties. In this study, the antianxiety effect of MPS skin was tested.

2. RESULTS

2.1. Light-dark box activity test (LDA)

There was a significant (P<0.05) reduction of time spent in a dark chamber in alprazolam (563.99 ± 8.37 s), tryptophan (531.83 ± 66.13 s), and 5-HTP (566.76 ± 23,07) group compared to CMC Na group (600 ± 0.00 s) (Figure 1). The decreased time in the dark chamber was also followed by the increased time in the light chamber (Figure 2). Rats in the alprazolam and 5-HTP group spent a comparable time duration in the light chamber, namely 36.01 ± 8.37 s and 33.24 ± 23.07 s. However, the tryptophan group spent the longest time in the light chamber (68.17 ± 66.13 s). Rats in the alprazolam, tryptophan, and 5-HTP group were also more frequently entering the light chamber, namely 1.5 ± 0.58, 2.5 ± 1.91, and 2 ± 1.41 times. All rats in CMC Na, BPE 140, and 280 mg/kg only spent time in a dark chamber and did not exit the dark chamber until the end of the experiment (Figure 1, Figure 3, Figure 4).

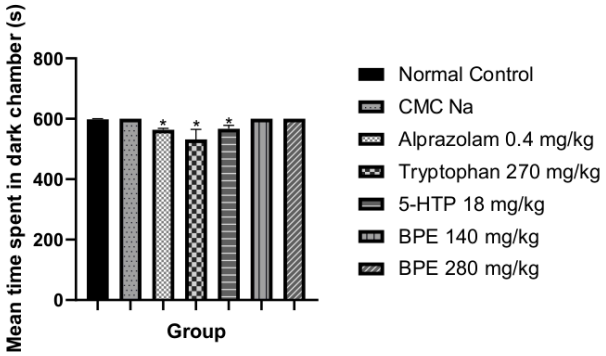

Figure 1. Comparison of dark box chamber time in the LDA test group. Data are expressed in means ± S.E.M. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

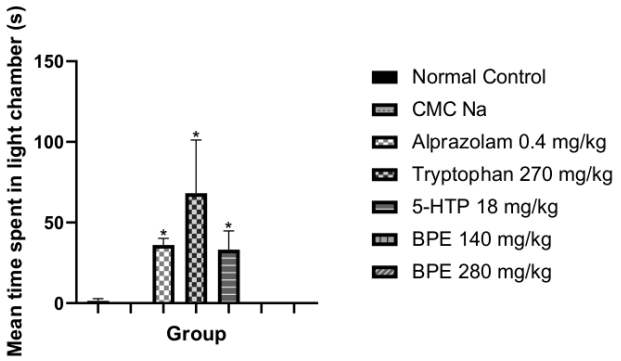

Figure 2. Comparison of light chamber time in the LDA test group. Data are expressed in means ± S.E.M. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

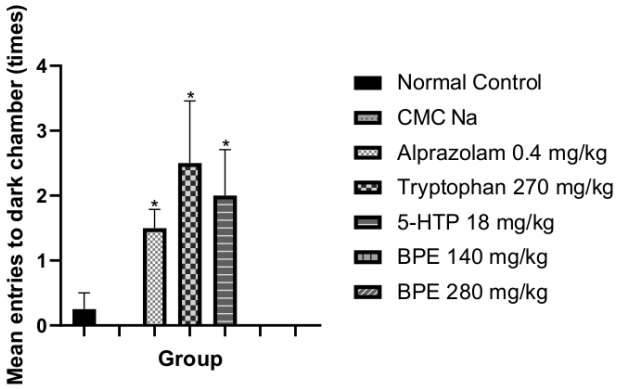

Figure 3. Comparison of entries to the dark chamber in the LDA test group. Data are expressed in means ± SEM. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

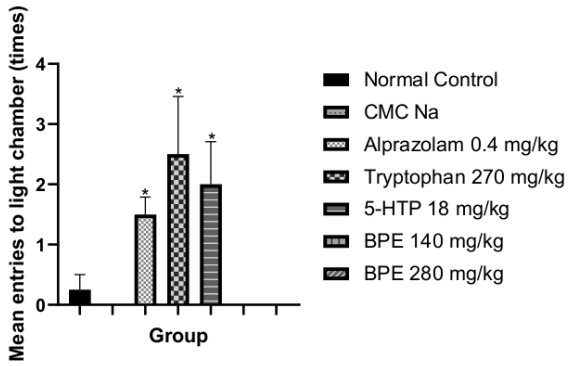

Figure 4. Comparison of entries to the light chamber in the LDA test group. Data are expressed in means ± S.E.M. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

2.1. Elevated Plus Maze (EPM)

The test results show a significant (P<0.05) decrease in the amount of time the rats spent in the closed arms (Figure 5) in the alprazolam group and 5-HTP group (185.99 ± 45.6 s and 207 ± 47.95 s) and an increase in the amount of time the rats spent in the open arms (Figure 6) (85.68 ± 39.40 s and 46.82 ± 19.62 s) compared to CMC Na group (spent 263.09 ± 30.59 s in the closed arms and 14.73 ± 6.01 s in the open arms). There was no significant difference between the groups regarding the number of entries into the closed arms (Figure 7). However, the entries into the open arms were significantly higher in alprazolam (5.83 ± 1.94 times), 5-HTP (3.16 ± 0.98 times), and BPE 280 mg/kg (2.67 ± 1.21 times) groups compared with CMC Na group (0.67 ± 0.82 times) (Figure 8). The increase in the number of entries into the open arms in the BPE 280 mg/kg group was not followed by an increase in the anxiety index (Figure 9).

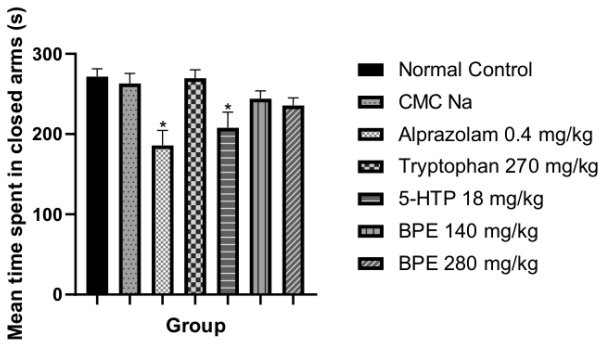

Figure 5. Comparison of closed arms time in the EPM test group. Data are expressed in means ± S.E.M. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

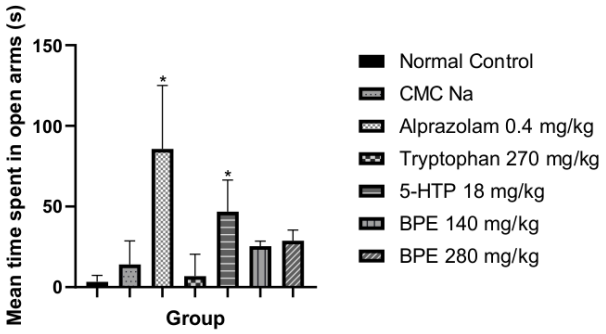

Figure 6. Comparison of open arms time in the EPM test group. Data are expressed in means ± S.E.M. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

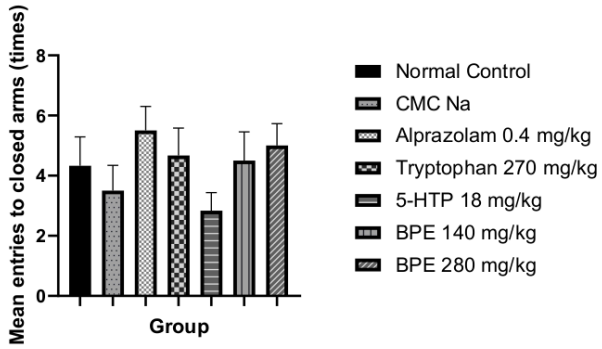

Figure 7. Comparison of entries to closed arms in the EPM test group. Data are expressed in means ± S.E.M. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

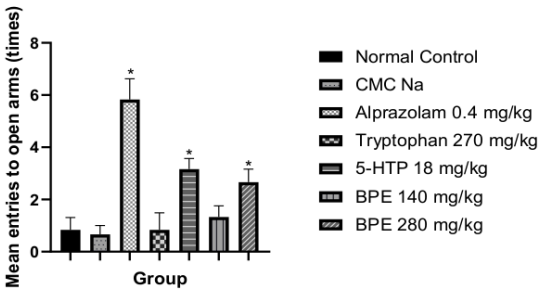

Figure 8. Comparison of entries to open arms in the EPM test group. Data are expressed in means ± S.E.M. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

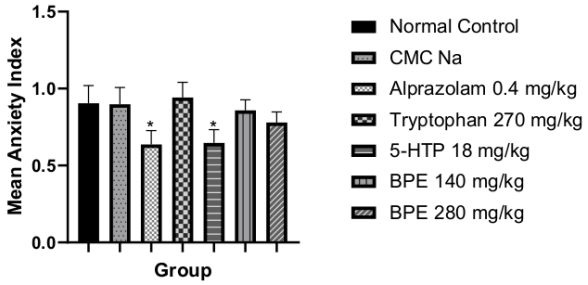

Figure 9. Comparison of anxiety index in the EPM test group. Data are expressed in means ± S.E.M. *P<0.05 against negative control (Kruskal-Wallis pairwise comparisons).

3. DISCUSSION

Based on the results of the LDA test, it can be concluded that tryptophan and 5-HTP may have anxiolytic properties. In contrast, BPE does not show this effect. The LDA test employs the ethological conflict between the rat's tendency to explore new environments and to avoid bright light in open areas. Anxiolytic drugs are expected to increase the transitions between light and dark spots. The number of transitions is highly correlated with exploratory behavior and is unrelated to locomotor activity in an undifferentiated open space [10]. The LDA test results in this study differ from previous studies, which showed significant differences in time in the light between rats treated with 70% acetone extract from the fruit pulp and peel of *Musa sapientum* [9]. In another study, giving *Musa paradisiaca* banana pulp could significantly increase the time spent in the light chamber [11]. In a previous study, tryptophan doses of 50 and 100 mg/ml/kg for 14 days increased the time spent in the light in rats induced with reserpine [12]. The tryptophan dose used in this study was much higher, i.e., 270 mg/kg, so a positive anxiolytic effect was also obtained. The body creates 5-HTP, an aromatic amino acid, from L-tryptophan, an essential amino acid. Since it was first synthesized for commercial usage from the seeds of the African plant *Griffonia simplicifolia*, 5-HTP has been utilized in therapeutic settings. The blood-brain barrier is easily crossed by 5-HTP, which efficiently stimulates serotonin production in the central nervous system (CNS). It is believed that taking 5-HTP supplements will restore serotonin production, which is thought to be connected to its antidepressant effects [13].

The results of the EPM test, represented by the anxiety index, show that apart from alprazolam, only 5-HTP exhibits anxiolytic effects. In the EPM test, the rats explored in closed and open arms, but the rats would more frequently enter and spend more time in closed arms. The preference percentage for open or closed arms, both the time and the number of entries, is called the anxiety index. The anxiety index was computed as follows, according to Cohen et al. [14], Anxiety Index = 1-[([Open arm time/Test duration] + [Open arms entries/Total number of entries])/2]. The more intense the anxiety, the lower the preference for open arms [15]. BPE 280 mg/kg shows an increase in the number of open-arm entries but does not show an increase in the anxiety index. In this case, it is more appropriate if the results of the anxiety index are used as a reference. The anxiety index combines data from each of the EPM parameters. The resulting ratio is an integrated parameter showing absolute size and overall trend [14]. Therefore, this parameter can be used as the primary reference in interpreting the EPM test results. It is known that several factors that can affect the results of the EPM test are gender, age, prior exposure or repeated exposure to EPM, time of the test, and room lighting. In addition, the way the rats were moved to the test room, placed in independent cages or shared cages, and where and how long the rats were kept in the room before the test was conducted are also some affecting factors [15].

The EPM test results differ from previous studies, which showed significant differences in time in the light between rats treated with 70% acetone extract from the fruit pulp and peel of *Musa sapientum* [9,16,17]. In each study, the doses used were 280 mg/kg and 420 mg/kg in rats for 14 and 16 days, respectively. This study used 50% ethanol extract, which was more polar compared to 70% acetone extract. The choice of solvent in this study is based on the physical and chemical properties of the target compound, in this case tryptophan. Previous research showed that the solubility of L-tryptophan was highest at ethanol concentrations of 49.96 – 75.08%, so 50% ethanol was chosen as the solvent in this study [18]. The results of previous studies showed that the tryptophan content in BPE was only 0.2%[19]. It is suspected that the choice of solvent affects the antianxiety effect of BPE, and optimization is needed to select extraction solvents.

An open-field test can measure anxiolytic effects other than LDA and EPM. One study reported that a 100% acetone extract dose of 200 mg/kg from ripe banana peels and unripe banana peels of *Musa paradisiaca* L. given for 21 days in Wistar rats could increase grooming, rearing, and box-crossing responses in the open field test [20]. The extract had a lower polarity index than 70% acetone or 50% ethanol and was administered at a lower dose but with a longer duration. However, previous studies used more polar solvents, such as 70% ethanol (polarity index 6.34). A 70% ethanol extract from *Musa paradisiaca* leaves at doses of 35, 70, and 140 mg/kg in rats given for seven days could also improve learning and memory abilities, characterized by decreased transfer time latency from open arms to closed arms [21]. In another study, a more polar solvent was also used. A 95% ethanol extract from the raw fruit of *Musa paradisiaca* L. at a dose of 500 mg/kg for five days gave positive anti-punishment and anti-frustration effects in male Wistar rats [22]. Banana stem water extracts with a dose of 17.5, 35, and 70 mg/kg in rats decreased time in closed arms and increased time spent in open arms when given 45 minutes before experimentation [23].

Giving banana pulp has been shown to have a positive antianxiety effect. Giving *Musa paradisiaca* banana pulp mixed in food (unmentioned dose) for 30 days can significantly increase the amount and length of time spent in open arms [11]. Previously, giving bananas to schizophrenic patients for 7 and 14 days was proven to reduce anxiety scores [24]. Based on these results, it is possible that the frequency of giving BPE only once also affected the absence of an anxiolytic effect.

Despite the many research results showing the anxiolytic effects of various parts of the banana, one study showed the opposite result. A non-significant increase in the time spent in the open arm of the EPM test was found after the administration of an aqueous extract from *Musa sapientum* leaves one hour before the test [25]. It shows that the choice of extraction solvent significantly affects the antianxiety effect. Although in the study, the tryptophan EPM test did not have a significant effect, in previous studies, tryptophan doses of 50 and 100 mg/ml/kg given for 14 days increased the time spent in open arms in rats induced anxiety with reserpine [12].

The rat's tendency to avoid open spaces is related to thigmotaxis compared to the fear of heights. Thigmotaxis is the rodent's innate avoidance behavior, increasing when the animal is under stress. It is characterized by the rodent's preference for seeking shelter rather than exposing themselves to open areas [26]. Anxiety involves a conflict between the urge to avoid and explore a threatening stimulus (e.g., an open or bright area on a test kit) [27].

It is suspected that the results of the LDA test are not 100% related to the results of the EPM test. Ramos (2008) stated that variations between tests can occur in the same pharmacological study because of different constructs between tests. Emotionality is not unidimensional but varies on several independent axes that can only be accessed through tests involving stressful stimuli (e.g., novelty, light, exposure, punishment). Clinical anxiety also comprises a cluster of different pathologies triggered by different environmental stimuli.

Categorizing specific animal tests for certain subtypes of clinical anxiety is difficult, so viewing the various tests as an overlapping construct is a better approach. In addition, behavior is also the result of genetic and environmental factors, and the current tests can only measure momentary anxiety [27]. Current behavioral tests only measure momentary anxiety, so they are inadequate for modeling persistent conditions in humans [28].

Previous studies proved that rats' preferences in dark and light places did not affect rat behavior in the EPM tool. Animals prefer dark compartments when their environment is new and potentially dangerous, so a more protected area is preferred. Results have been inconsistent in nocturnal animals, such as rats, which prefer to be in the dark during the day if given the opportunity[15].

The validity of the tests currently available is highly dependent on the sensitivity of these tests to drugs that have been shown to have clinical effects, namely the benzodiazepine (BDZ) class of drugs. BDZ is considered the gold standard of reference compounds for new anxiolytic compounds. Apart from BDZ, there are also non-BDZ anxiolytic drugs, such as buspirone, a 5-HT1A agonist. Current anxiety tests have not been able to accommodate these new drugs. In addition, fluoxetine, an SSRI, also shows inconsistent and contradictory test results (anxiolytic and anxiogenic). In addition, current tests cannot distinguish between anxiolytic and psychostimulant effects [27].

The differences between state-induced anxiety and innate anxiety support the insensitivity of anxiety tests in animals to non-BDZ group compounds. Anxiety due to circumstances is found in certain situations (normal anxiety), while innate anxiety is an individual's tendency to experience anxiety in a chronic context and various situations (pathological anxiety). The anxiolytic drugs may overcome one but not all anxiety conditions.

4. CONCLUSION

Ethanol extract of *Musa paradisiaca* L. var Semeru did not show a significant effect as antianxiety as LDA and EPM tests have shown. It can be concluded that ethanol extract of *Musa paradisiaca* L. var Semeru did not show a significant effect as antianxiety. Other anxiety test techniques that are more sensitive to non-BDZ family medications must be used. To do this, new anxiety test techniques beyond those already available must be developed. Biomarker assessments must support the findings of behavioral studies on anxiety disorders.

5. MATERIALS AND METHODS

5.1. Materials

Unripe banana peels were obtained from farmers in Burno Village and Jambearum Village, Lumajang Regency, Indonesia. Ir. Paulina Evy Retnaning Prahardini, MP, who released the Semeru variety, identified and authenticated the plant referred to the Decree of the Ministry of Agriculture of the Republic of Indonesia No. 352/Kpts/LB.240/6/2004, Alprazolam (PT. Otto Pharmaceutical Industries, Indonesia), L-tryptophan (Swanson, USA), 5-HTP (Now, USA).

5.2. Preparation of Banana Peel Powder

Banana peels were chopped, powdered, then dried in an oven at 70 °C after being cleaned with tap water and then distilled water. It was sieved with a 40-mesh sieve after being dried and pounded into a fine powder. Before extraction, it was kept as a dry powder in storage.

5.3. Extraction of Banana Peel Powder

Banana peel powder was extracted using the maceration process at room temperature and 50% ethanol as the solvent. One thousand grams of powdered banana peel were steeped in 2.5 liters of 50 % ethanol for 24 hours while occasionally being stirred. The filtered paper was used to filter the suspension, and one liter of 50% ethanol was added to the residue. Maceration was repeated seven times using the same process. The concentrated sample was kept in a water bath as the solvent evaporated.

5.4. Laboratory animals

Seventy male albino Wistar strain rats (*Ratus novergicus*), eight weeks old and weighing about 118 grams, were used in this study (@4 rats per group for the LDA test and @6 rats per group for the EPM test). Rats were procured from a local veterinarian breeder in Surabaya, transported to the animal laboratory, and kept at 25 to 27 °C and a humidity of 75%. The rats underwent a week of acclimatization before being utilized in the investigation. Special rat pellets (PT. Indofeed, Indonesia) and mineral water were freely offered to the

rats. Rats were weighed for body weight, and their behavior was recorded to assess their health. If an animal does not exhibit any signs of illness and loses no more than 10% of its initial weight, it is considered healthy and can be employed for the study. This research has received approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing at Gadjah Mada University, Indonesia, with the reference number KE/FK/1133/EC/2022.

5.5. Test for anxiolytic activity

5.5.1. Light dark box activity test (LDA)

The test equipment is made locally according to the light-dark box design produced by MazeEngineers and Panlab. The apparatus consists of an opaque dark chamber (20x31x40 cm; 2 lux) and a light chamber (31x31x40 cm; 390 lux) with access (10x10 cm) between them. The test protocol was implemented based on the previous protocol [10,29]. All test rats were brought to the room once the equipment was set up. They waited for up to 60 minutes while receiving sound stimulation from a white noise generator, the volume of which was higher than the ambient noise. The acute test was conducted by giving the test compound orally to all experimental groups, except the normal control group, 60 minutes before the test. The test compounds are banana peel extract (BPE) 140 and 280 mg/kg; tryptophan 270 mg/kg, 5-HTP 18 mg/kg; alprazolam 0.4 mg/kg and carboxymethylcellulose (CMC) Na 0.5% for the control group. At the start of the test, each rat was placed in a dark chamber. The partition between the chambers was closed for 3 seconds and then opened. The rats' activity exploring the dark and light chambers was recorded for 10 minutes. After each experiment was completed or after each rat change, the chamber was cleaned with 70% alcohol and then with water. The rats were then left for 10 minutes. The number of rat transitions between compartments and the time spent in each compartment was calculated.

5.5.2. Elevated plus maze test (EPM)

Local manufacturers construct the test apparatus following Panlab's elevated plus maze design. Two identically sized open and closed arms make up the test device (10 x 100 cm). The wall of the closed arm is 50 cm high. A central square (10 x 10 cm) connects the two arm kinds to create a plus symbol. The maze has a 65 cm height. After the test room's equipment was set up, all test rats were brought in and waited for up to 60 minutes while receiving sound stimulation from a white noise generator, the volume of which was higher than the ambient noise. The acute test was performed by administering the test substance orally to all experimental groups, except the normal control group, 60 minutes before placing the rats in the maze. The test compounds are banana peel extract (BPE) 140 and 280 mg/kg; tryptophan 270 mg/kg, 5-HTP 18 mg/kg; alprazolam 0.4 mg/kg and carboxymethylcellulose (CMC) Na 0.5% for the control group. Each rat was placed right in the middle of the maze with the rat's head facing the open arm. The activity of rats exploring EPM freely for 5 minutes was recorded. After each experiment, the equipment was cleaned with 70% alcohol and water. The number of entries and time in the open and closed arms was calculated.

5.5.3. Data Analysis

SPSS version 26 for Windows was used for statistical analysis. The normality of data distribution was tested using the Shapiro-Wilk test, followed by a comparison test using one-way ANOVA when the data distribution was normal; otherwise, the Kruskal-Wallis test was used.

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Author contributions: Conception – I.S., I.P.; Design – I.S., I.P.; Supervision – I.P., A.P.G., L.H.; Resources – I.S.; Materials – I.S.; Data Collection and Processing – I.S.; Analysis and Interpretation – I.S., I.P.; Literature Research – I.S.; Writing Manuscript – I.S., I.P., A.P.G., L.H.; Critical Review – I.S., I.P., A.P.G., L.H.

Conflict of interest statement: The authors declared no conflict of interest.

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