Effect of Angiotensin Receptor Blocker and Probiotics in Caffeine-Induced Anxiety Model

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ABSTRACT

Aim: To evaluate anti-anxiety effect of probiotics and angiotensin receptor blocker in caffeine induced anxiety rat model.

Methodology: The study was conducted in six groups (n=6). Caffeine was given intraperitoneally at a dosage of 25 mg/kg each day for 14 days. Blood samples, body temperature, and glucose measurements were taken prior to the trial. Caffeine was given intraperitoneally to G1-G5 mice at a dosage of 25 mg/kg. For groups 3 and 4, a probiotic formulation was given orally following the caffeine dosage. In group 5, a probiotic topical gel (30 mg) was given topically to the animals' foreheads. Diazepam (1 mg/kg) was given to group 2 animals.

Results: The body weight was not changed significantly throughout the study period in all the groups. Mortality and clinical signs were not observed during study. When compared to the anxiety control group, there was no statistical significance in either of the groups' glucose levels. There was no statistical significance in Hemoglobin (Hb) levels of animals on day 14 when we compare with anxiety control group. The changes in SGOT and SGPT levels were not found to be statistically significant. There was no statistical significance in rectal temperature of animals. There was no statistical significance in all the groups in hot plate readings on day 7 when compared with anxiety control group. Whereas there was significance increase in latency time (time spent) was observed in group 2 & group 6 when compared to anxiety control group on day 14. Probiotic high dosage and probiotic topic gel treated animals similarly spent significantly more time in open arms than anxiety

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control animals (P< 0.01). High serotonin levels were observed in the anxiety control rats, however it reduced in treatment groups.

**Conclusion:** The study concludes the potential benefit of probiotics and Losartan for the treatment of caffeine induced anxiety.

**Keywords:** Probiotics; losartan; anxiety; caffeine; angiotensine.

**1. INTRODUCTION**

The most frequent mental abnormalities in human health are depression and anxiety disorders. Anxiety is an emotional condition characterised by tension, worry, and dread, as well as physiological changes such as palpitations, tremors, gastrointestinal, respiratory, and circulatory issues that have no obvious objective etiology. According to the World Health Organization (WHO), the global prevalence of depression was as high as 4.4% in 2015, while the prevalence of anxiety disorders was estimated to be 3.6% [1]. According to current National Institute of Mental Health data, 18.1% of individuals in the United States are afflicted, with 22.8% of instances deemed severe. The average age of start of anxiety is 11 years old, and women are 60% more likely than males to be afflicted [2]. Anxiety disorders were placed sixth among all diseases in the global population due to their high frequency and devastating characteristics. Excessive anxiety is linked to several unfavorable health outcomes, including an increased risk of coronary heart disease and sleep disturbances [3].

Probiotics are live bacteria that attempt to improve health by rebuilding and maintaining the gut flora. The increasing function of probiotics in medical science is a new field of study. Recent research reveals that gut microorganisms alter physiological aspects in the body and contribute to inflammation and mental illnesses [4]. According to extensive research, gut microorganisms communicate with the central nervous system via the gut-brain axis. Probiotic-based therapy has been offered as a potential treatment for several neurological and neurodegenerative illnesses because probiotics influence the host gut microbial populations and their synthesis/release of specific neuroactive chemicals [5]. Some probiotic strains have been demonstrated in clinical tests to aid with stress, anxiety, and depression symptoms [6].

It has been claimed that under stressful situations, the brain and angiotensin II activate the AT1 receptor in the brain, causing the release of hormones such as CRH, ACTH, corticosteroids, and vasopressin, as well as stimulating sympathetic activity (fight and fear activities). Angiotensin II receptor blockers are compounds with a low risk of side effects. They are used to treat hypertension and heart failure because of their anti-inflammatory qualities [7]. Stress-induced decreases in cortical CRH(1) and benzodiazepine binding are prevented by AT(1) receptor antagonists, which are anxiolytic. Angiotensin II AT(1) receptor blockers like Losartan in the brain might be a new therapy option for anxiety and other stress-related diseases. The Losartan in few of the studies was found to be effective in the management of the anxiety related disorders [8]. Losartan has been shown to accelerate fear extinction in animals and to prevent the development of anxiety disorders in humans, emphasising its potential to possibly enhance exposure therapy in humans [7,8].

Caffeine stimulates the sympathetic nervous system, which stimulates noradrenergic neurons and influences dopamine release locally [9]. Anxiogenic neurotransmission is caused by dopaminergic neurotransmission. Caffeine is the most widely used stimulant, and it possesses anxiogenic properties. Anxiety symptoms differ from person to person depending on their sensitivity to the methylxanthine component found in coffee [10]. Because of the hazards involved with synthetic anti-anxiety medicines, natural alternatives that are free of side effects are recommended.

Anxiety requires more therapy than depression, according to experts. Only a small percentage of patients believe they require medicine or counseling, and the majority of instances go undetected [11]. There is a significant disparity in severity rates, which leads to disparities in consumption rates. There’s also a potential that the symptoms are misinterpreted as a general ailment rather than a mental condition. As a result, particular diagnostic tools and criteria for service demand are required. Our research group wanted to see if probiotics and angiotensin receptor blockers have anti-inflammatory effects.
on the brain. Chronically high levels of inflammation in the body and brain are now recognised as one of the main underlying causes of anxiety and other mental illnesses.

2. MATERIALS AND METHODS

2.1 Materials

Caffeine, Diazepam, Losartan and Probiotic were purchased from Sigma Aldrich. All other chemicals were of analytical grades used in the study.

2.2 Animal Handlings and Maintenance

Wistar male rats weighing 180 to 220 gm (6 to 8 weeks old) were purchased from Adita biosystems. All animal handlings were performed as per ethical practices laid down in the CPCSEA guidelines [1] for animal care and use. The study was approved by the Institutional Animals Ethics Committee (IAEC) of the test facility (IAEC SSCP No: 171/2020-21). The animals were acclimatized for a minimum period of 7 days to laboratory conditions and will be observed for clinical signs daily. The animals were housed under standard laboratory conditions, air-conditioned with adequate fresh air supply (Air changes 12-15 per hour), room temperature 20.2 to 22.7°C, relative humidity 49-63%, with 12 hours light and 12 hours dark cycle. Each animal was housed in an individual cage with stainless steel mesh top grill having facilities for holding pellet food and drinking water. The animals were fed with standard laboratory rodents and reverse osmosis drinking water was provided ad libitum.

2.3 Induction of Anxiety and Treatment

The study was performed in 6 groups of animals each containing 6 in each group (n=36). Animals were acclimatized for seven days before the study. Caffeine & Diazepam was dissolved in 0.9% normal saline. Probiotic was formulated in sterile water and dosed as shown in the experimental design. Caffeine was dosed at 25 mg/kg intraperitoneally daily for 14 days. Pre-study blood samples, body temperature & glucose readings were recorded. On day 8 before treatment begins, all groups of animals were weighed. Group-6 animals were administered with vehicle (normal saline). G1-G5 animals were dosed with caffeine at a dose of 25 mg/kg intraperitoneally. The probiotic formulation was administered orally after the caffeine dose for groups 3 & 4. Probiotic topical gel (30 mg) was applied topically over the forehead of animals in group 5. Group 2 animals were treated with diazepam (1 mg/kg). The dose-volume for oral dosing was 10 ml/kg and intraperitoneal dosing was 5 ml/kg. The grouping of animals and treatment is presented in Table 1.

2.4 Pain Test by Eddy’s Hot Plate Method

The animals’ basal response time was measured by exposing them to a hot plate that was kept at a constant temperature of 55°C. Any type of initial reaction, like jumping or paw licking, was observed and recorded. To minimise causing injury to the paws, a 15-second cut was proposed. This test was done both before (Day 7) and at the end of the treatment (14 Day) [12].

2.5 Elevated Plus Maze test (EPM)

On days 7 and 14, rats were tested in an elevated plus-maze. The light phase of the light/dark cycle was used for all of the tests. Each session lasted 5 minutes and was conducted with the lights turned on. Each test started with the rat facing the maze’s junction. To avoid the introduction of pheromonal signals, the EPM was cleaned promptly after each test. The timer was used to manually record exploratory behaviour.

Table 1. Grouping of the animals and treatment details

| Group | Group ID         | Treatment                                      | Total No. of animals |
|-------|------------------|------------------------------------------------|----------------------|
| 1     | Anxiety Control  | Caffeine 25 mg/kg, i.p                         | 6                    |
| 2     | Anxiety + Diazepam | Caffeine 25 mg/kg, i.p & diazepam              | 6                    |
| 3     | Anxiety + Probiotic dose | Caffeine 25 mg/kg, i.p+ Probiotic dose 1 mg/kg | 6                    |
| 4     | Anxiety + Losartan | Caffeine 25 mg/kg, i.p+ Losartan 10 mg/kg     | 6                    |
| 5     | Anxiety + Probiotic topical gel | Caffeine 25 mg/kg, i.p+ Probiotic topical gel (30 mg on fore head) | 6                    |
| 6     | Normal Control   | saline, i.p                                     | 6                    |
on the maze. The dependent measurements were basic movements, open arm entries, and open/closed arm time [13].

2.6 Body Weight and Rectal Temperature Measurement

Body weights were recorded on the day of the experiment start and weekly once during the treatment period. Animals were checked for rectal temperature using a rectal thermometer before (Day 7) & at end of the treatment (Day 14).

2.7 Clinical Signs and Clinical Parameters

Clinical signs were recorded daily from the start of the experiment to the end of the experiment. Whole blood (Hb) was determined using an automated clinical analyser. Serum SGOT and SGPT was determined using a clinical analyser. Animals were checked for fasting blood glucose using a commercially available glucometer on (Day 7) & at end of treatment (Day 14) [14,15].

2.8 Serotonin Levels in Blood Serum

3.25 ml of blood was withdrawn from test animals and added to anticoagulant-free tubes and centrifuged at 3500 rpm for ten minutes at 5 degrees to obtain serum. The serum aliquots were frozen and stored at -80°C until the day of the analysis. The serotonin was measured from the ELISA Serotonin kit. Normal serum serotonin levels should be 50-200 ng/ml.

2.9 Statistical Analysis

The Mean ± SD Body weight and rectal temperature were estimated in each group. SGOT, SGPT, Hb, glucose readings, EPM & Eddy’s hot plates readings were estimated in each group and SD was determined. Significant differences between group mean and control were analyzed by one-way ANOVA, followed by a Dunnett’s multiple comparison test, using Graphpad Prism at 95% confidence levels. P-value of <0.05 was considered significant.

3. RESULTS AND DISCUSSION

In present study rats were employed as an animal model to assess the anti-anxiety effects of probiotics and angiotensine receptor (AT) antagonist i.e. Losartan. In order to produce anxious behaviour in rats, a standardised dosage of caffeine of 25 mg/kg was utilised. Caffeine has long been recognised to increase anxiety in people and animal models. Losartan, probiotic, and probiotic topical gel doses were compared to the efficacy of the well-known anti-anxiety drug diazepam in a caffeine model. The research was conducted over a 14-day period. Body weight, clinical signs, blood glucose level, clinical chemistry (SGOT, SGPT), rectal temperature, eddy’s hot plate and elevated plus maze (EPM) was the parameters assessed over the study period [16,17].

3.1 Body Weight

The body weight was not changed significantly throughout the study period in all the groups of the animals. The body weights of all the animals in each group are presented in Table 2 and Fig. 1. Anxiety control rats had a higher body weight than normal control rats because anxiety control rats had a higher appetite and were likely eating more. The body weight, on the other hand, decreased following the therapy.

3.2 Mortality and Clinical Signs

During the entire period of the study the mortality and clinical signs were not observed. It confirmed that the administered dose of all the test and control samples were well tolerated by the animals. These observations proved the safety of the probiotics and losartan. The observations are presented in Table 3.

| Group ID | Group ID ID | Bodyweight (g) (Mean ± SD) |
|----------|-------------|---------------------------|
|          |             | Day 1 | Day 7 | Day 14 |
| 1         | Anxiety Control | 190.0±5.2 | 196.0±5.1 | 199.4±5.2 |
| 2         | Anxiety + Diazepam | 192.4±3.2 | 198.4±3.2 | 202.2±4.0 ns |
| 3         | Anxiety + Probiotic dose | 194.0±3.6 | 200.0±3.8 | 204.4±4.5 ns |
| 4         | Anxiety + Losartan | 192.0±5.8 | 198.4±5.9 | 201.8±5.1 ns |
| 5         | Anxiety + Probiotic topical gel | 193.6±4.7 | 199.2±4.3 | 202.6±3.3 ns |
| 6         | Normal Control | 191.2±5.0 | 197.6±5.1 | 202.2±5.3 ns |

ns: Statistically non-significant when compared to vehicle group [p>0.05]
The comparative body weights of all the animals in each group

![Graph showing body weight Day 14](image)

**Fig. 1. The comparative body weights of all the animals in each group**

Table 3. No clinical signs for all the animals throughout the experiment period

| Group       | Group ID            | Clinical signs       | Day 1-7  | Day 8-14 |
|-------------|---------------------|----------------------|----------|----------|
| 1           | Anxiety Control     | N                    | N        | N        |
| 2           | Anxiety + Diazepam  | N                    | N        | N        |
| 3           | Anxiety + Probiotic dose 1 | N                  | N        | N        |
| 4           | Anxiety + Losartan  | N                    | N        | N        |
| 5           | Anxiety + Probiotic topical gel | N                  | N        | N        |
| 6           | Normal Control      | N                    | N        | N        |

*N: Normal*

3.3 Glucose Levels

When compared to the anxiety control group, there was no statistical significance in either of the groups’ glucose levels on day 7. Whereas in group 1 there was a significant rise in glucose levels (perhaps owing to an increase in anxiety levels), glucose readings in the other groups remained stable (i.e., G2, G4, G5 & G6). The glucose levels on 7 th and 14 th day in all animal groups are presented in Table 4 and graphically shown in Fig. 2. The glycemic index of anxiety control rats was higher because they experienced an insulin rise due to increased appetite/high carbohydrate consumption [18].

The glucose levels, on the other hand, were lower following the therapy.

3.4 Hemoglobin (Hb) Level

There was no statistical significance in Hemoglobin (Hb) levels of animals on day 14 when we compare with anxiety control group as shown in Table 5 and Fig. 3. Hb levels was found lower in anxiety control rats. Because many people who suffer from anxiety hyperventilate, a huge percentage of them may be contributing to a magnesium deficit and, as a result, anaemia. The Hb levels, on the other hand, improved following the therapy.

Table 4. Comparative glucose levels in all the animals throughout the experiment period

| Group       | Group ID           | Glucose Readings (mg/dl) | Day 7       | Day 14      |
|-------------|--------------------|--------------------------|-------------|-------------|
| 1           | Anxiety Control    | 96.83±4.30               | 112.67±12.05|
| 2           | Anxiety + Diazepam | 95.67±3.35<sup>ns</sup> | 97.00±2.71<sup>**</sup>|
| 3           | Anxiety + Probiotic dose 1 | 96.33±3.14<sup>ns</sup> | 103.00±8.35<sup>ns</sup>|
| 4           | Anxiety + Losartan | 96.50±2.93<sup>ns</sup> | 97.50±3.10<sup>**</sup>|
| 5           | Anxiety + Probiotic topical gel | 95.67±3.40<sup>ns</sup> | 96.50±3.95<sup>**</sup>|
| 6           | Normal Control     | 95.33±5.25<sup>ns</sup> | 95.83±4.30<sup>**</sup>|

*ns: Statistically non-significant when compared to vehicle group [p>0.05], **: statistically significant*
**Table 5. Hemoglobin (Hb) levels of animals on day 14 when we compare with anxiety control group**

| Group ID | Group | Hemoglobin (g/dl) at 14 D |
|----------|-------|--------------------------|
| 1        | Anxiety Control  | 12.0±0.7                 |
| 2        | Anxiety + Diazepam| 11.8±0.6\textsuperscript{ns} |
| 3        | Anxiety + Probiotic dose | 12.0±0.3 \textsuperscript{ns} |
| 4        | Anxiety + Losartan | 12.0±1.0 \textsuperscript{ns} |
| 5        | Anxiety + Probiotic topical gel | 11.8±0.6 \textsuperscript{ns} |
| 6        | Normal Control    | 12.2±0.6 \textsuperscript{ns} |

\textsuperscript{ns}: Statistically non-significant when compared to vehicle group \([p>0.05]\)

**3.5 SGOT and SGPT Levels**

The levels of clinical parameters like SGOT and SGPT are presented in Table 6 and Fig. 4. The anxious control rats had greater liver enzymes than the treatment and reference drug animals (diazepam). The majority of these coagulation factors are produced by the liver. As a result, increased liver levels (SGPT, SGOT) caused by anxiety can raise blood pressure, exerting additional stress on the blood vessel walls, stiffening them and reducing the volume of blood flowing through the body. When these factors come together, they can cause dangerous blood clots.

**3.6 Rectal Temperature**

There was no statistical significance in rectal temperature of animals on day 7 and day 14 when we compare with anxiety control group (Table 7 and Fig. 5). Anxiety may induce changes in the heart rate and blood circulation throughout the body, therefore raising the temperature can help with anxiety control [19]. Increased blood flow delivers new oxygen and nutrients to the muscles, while a higher heart rate makes it easier to run or fight. Vasoconstriction occurs when blood vessels narrow, and it can impact body temperature, resulting in a fever [19]. The rectal temperature levels, on the other hand, improved following the therapy.
Table 6. SGOT and SGPT levels in animal groups

| Group               | Group ID | Clinical Chemistry (Mean±SD) | SGOT(IU/L) | SGPT(IU/L) |
|---------------------|----------|------------------------------|------------|------------|
| 1 Anxiety Control   |          |                              | 49.8±4.6   | 52.5±2.6   |
| 2 Anxiety + Diazepam|          |                              | 45.3±2.1 ns| 45.0±2.3   |
| 3 Anxiety + Probiotic dose 1 |          |                              | 47.0±1.9 ns| 45.5±1.9   |
| 4 Anxiety + Losartan |          |                              | 45.8±2.3 ns| 41.8±2.5   |
| 5 Anxiety + Probiotic topical gel |          |                              | 46.0±3.9 ns| 42.2±2.1   |
| 6 Normal Control    |          |                              | 45.2±2.3 ns| 42.7±2.4   |

Fig. 4. The SGPT and SGOT levels on 14th day in all animal groups

Table 7. Rectal temperature of animals on day 7 and day 14 when we compare with anxiety control group

| Group               | Group ID | Rectal temperature (°C) | Day 7 | Day 14 |
|---------------------|----------|--------------------------|-------|--------|
| 1 Anxiety Control   |          |                          | 36.92±0.73 | 37.10±0.29 |
| 2 Anxiety + Diazepam|          |                          | 36.67±0.80 ns| 36.47±0.96 ns |
| 3 Anxiety + Probiotic dose 1 |          |                          | 36.45±0.72 ns| 36.17±0.27 ns |
| 4 Anxiety + Losartan |          |                          | 36.85±0.39 ns| 36.42±0.65 ns |
| 5 Anxiety + Probiotic topical gel |          |                          | 36.67±0.54 ns| 37.00±0.60 ns |
| 6 Normal Control    |          |                          | 37.07±0.26 ns| 36.58±0.46 ns |

3.7 Eddys Hot Plate Study

There was no statistical significance in all the groups in hot plate readings on day 7 when compared with anxiety control group. Whereas there was significance increase in latency time (time spent) was observed in group 2 & group 6 when compared to anxiety control group on day 14 (Table 8 and Fig. 6). Anxiety control rats could only stay on Eddy’s hot plate for a short period. Higher levels of anxiety are linked to a lower perceived pain threshold and more acute pain perceptions, which explains why they only lasted a few minutes. The treatment group, on the other hand, stayed on the plate for a longer period of time.

3.8 Elevated Plus Maze Test (EPM)

Animals given diazepam (1 mg/kg) spent substantially more time in open arms than anxiety control mice (P< 0.01). Probiotic high dosage and probiotic topical gel treated animals similarly spent significantly more time in open arms than anxiety control animals (P< 0.01). When compared to the anxiety control group, the probiotic low dosage group was non-significant (Table 9 and Fig. 7). An increase in the
The proportion of time spent in the open arms (time in open arms/total time in open or closed arms) and an increase in the proportion of entries into the open arms (entries into open arms/total entries into open or closed arms) suggest anxiety decrease in the plus-maze. The anxiety control rats exhibited higher anxiety than the treatment animals.

![Graph showing rectal temperature on 7th and 14th day in all animal groups](image1)

**Fig. 5. The rectal temperature on 7th and 14th day in all animal groups**

**Table 8. Comparative eddys hot plate study results on day 7 and day 14 when we compare with anxiety control group**

| Group                     | Group ID | Hot plate readings (Seconds) | Day 7   | Day 14   |
|---------------------------|----------|------------------------------|---------|----------|
| 1  Anxiety Control        |          |                              | 7.17±0.69 | 7.75±0.69 |
| 2  Anxiety + Diazepam     |          |                              | 6.50±0.76 ns | 13.50±0.96 *** |
| 3  Anxiety + Probiotic    |          |                              | 7.00±0.58 ns | 10.17±2.27 ns |
| 4  Anxiety + Losartan     |          |                              | 7.33±1.25 ns | 7.33±3.14 ns |
| 5  Anxiety + Probiotic gel|          |                              | 7.33±0.94 ns | 5.83±1.07 ns |
| 6  Normal Control         |          |                              | 7.83±0.69 ns | 4.00±0.58 * |

![Graph showing hot plate study results on 7th and 14th day in all animal groups](image2)

**Fig. 6. Comparative eddys hot plate study results on 7th and 14th day in all animal groups**
Table 9. Comparative time spent in open arm (seconds) on day 7 and day 14

| Group ID | Time spent in open arm (seconds) |
|---------|----------------------------------|
|          | Day 7 | Day 14 |
| 1 | Anxiety Control | 63.7 ±18.6 | 73.3 ±13.2 |
| 2 | Anxiety + Diazepam | 63.3±2.7<sup>ns</sup> | 205.8±43.3<sup>***</sup> |
| 3 | Anxiety + Probiotic dose 1 | 65.0±10.5<sup>ns</sup> | 109.8±28.3<sup>ns</sup> |
| 4 | Anxiety + Losartan | 60.0±17.9<sup>ns</sup> | 143.0±16.6<sup>***</sup> |
| 5 | Anxiety + Probiotic topical gel | 61.7±20.4<sup>ns</sup> | 179.0±28.1<sup>***</sup> |
| 6 | Normal Control | 75.0±19.7<sup>ns</sup> | 82.7±15.7<sup>ns</sup> |

Fig. 7. Comparative time spent in open arm (seconds) on 7th and 14th day in all animal groups

3.9 Serotonin Levels in Blood Serum

Serotonin is a chemical messenger that is thought to regulate mood. It's supposed to aid in the production of good sleeping patterns and to improve your mood. Serotonin levels can affect mood and behaviour, according to extensive research it has been confirmed that the neurotransmitter is generally associated with feeling good and living longer. Too much serotonin, on the other hand, can be hazardous and even fatal. Serotonin syndrome is a disorder caused by elevated levels of serotonin in the body caused by certain drugs [20]. High serotonin levels were observed in the anxiety control rats however it reduced in treatment groups.

Fig. 8. Comparative time spent in open arm (seconds) on 7th and 14th day in all animal groups
| A.No. | Anxiety control | Anxiety+Diazepam (1 mg/kg) | Anxiety+Probiotic dose | Anxiety+Losartan | Anxiety+Probiotic topical gel | Normal control |
|-------|-----------------|-----------------------------|------------------------|-----------------|-------------------------------|----------------|
| 1     | 250             | 100                         | 221.1                  | 215.7           | 220.9                         | 97.35          |
| 2     | 245             | 93                          | 216.5                  | 200.17          | 221.85                        | 85.21          |
| 3     | 252             | 95                          | 225.12                 | 202.4           | 218.75                        | 76.6           |
| 4     | 260             | 100.2                       | 222.47                 | 208.97          | 222.87                        | 71             |
| 5     | 257.3           | 115.2                       | 225.18                 | 215.91          | 217.6                         | 75.21          |
| 6     | 259             | 100.37                      | 220.49                 | 214.12          | 220.13                        | 76.34          |
| Mean  | 253.8           | 100.6                       | 221.8                  | 209.5           | 220.4                         | 80.3           |
4. CONCLUSION

We may infer from the overall research that Losaratan’s involvement resulted in a considerable reduction in anxiety when compared to diazepam. However, in rats with caffeine-induced anxiety, probiotic oral and probiotic gel demonstrated a slight to moderate antianxiety benefit. As a result, probiotic oral and probiotic gel can be used to treat mild to moderate anxiety and can be used to substitute anti-anxiety drug adverse effects.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animal handlings were performed as per ethical practices laid down in the CPCSEA guidelines [1] for animal care and use. The study was approved by the Institutional Animals Ethics Committee (IAEC) of the test facility (IAEC SSCP No: 171/2020-21).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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